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# SPEEDTOOLS TISSUE DNA EXTRACTION KIT

***Kit for the Isolation of Genomic DNA from Tissue, Cells, Bacteria, Yeast and Body Fluids***

## Instructions for Use

**(Ref. 21.135M/6/7)**

***PLEASE READ THE INSTRUCTIONS FOR USE THOROUGHLY BEFORE USING THE KIT, ESPECIALLY IF YOU ARE NOT FAMILIAR WITH THE PROTOCOL.***

# Table of Contents

## 1. BASIC PRINCIPLE

**SPEEDTOOLS TISSUE DNA EXTRACTION KIT** is designed for the rapid isolation of highly pure genomic DNA from: **tissue**, **cells** (e.g. **bacteria, yeast**), **paraffin embedded samples**, **urine**, **feces**, dried **blood** spots and many other sources.

Lysis is achieved by incubation of the sample material in a proteinase K / SDS solution. Afterwards the lysate is treated with chaotropic ions and molecular grade ethanol to create the appropriate conditions for binding of DNA to the silica membrane of the column. The binding process is reversible and specific to nucleic acids. The washing steps efficiently remove contaminations and pure genomic DNA is finally eluted under low ionic strength conditions in a slightly alkaline elution buffer.

## 2. KIT CONTENTS

| SPEEDTOOLS TISSUE DNA EXTRACTION KIT | | | |
|---|---|---|---|
| **REAGENTS** | **10 Preps Ref. 21.135M** | **50 Preps Ref. 21.136** | **250 Preps Ref. 21.137** |
| Buffer BT1 | 5 ml | 20 ml | 5 x 20 ml |
| Buffer BB3 | 3 ml | 15 ml | 5 x 15 ml |
| Buffer BB5 (concentrate) | 3 ml | 12 ml | 5 x 12 ml |
| Buffer BBW | 6 ml | 30 ml | 5 x 30 ml |
| Buffer BBE | 3 ml | 13 ml | 5 x 13 ml |
| Proteinase K (lyophilized) | 6 mg | 30 mg | 5 x 30 mg |
| Proteinase Buffer (PB) | 400 µl | 1.8 ml | 5 x 1.8 ml |
| Speedtools Tissue Columns (plus Collection Tubes) | 1 x 10 | 50 | 5 x 50 |
| Collection Tubes | 1 x 20 | 100 | 5 x 100 |

## 3. INTENDED USE

With the **SPEEDTOOLS TISSUE DNA** method, total DNA (genomic and mitochondrial) is prepared from tissue, cells and many other sources. It is also suitable for purify bacterial and viral DNA from these samples.

One Speedtools Tissue Column is capable of binding up to 60 µg of genomic DNA. It allows isolation of up to 35 µg of highly pure genomic DNA with an A260/280-ratio between 1.60 and 1.90.

For lysis of certain bacterial and yeast strains additional enzymes not included in the kit may be necessary. Relevant support protocols are provided with this manual.

The obtained DNA is ready to use for subsequent reactions like PCR, Southern blotting, or any kind of enzymatic reactions.

| Table 1: General Characteristics of the Kit | |
|---|---|
| Sample material | □ 25 mg tissue/ $10^2$-$10^7$ cultured cells |
| Expected yield | 20-35 µg |
| Elution volume | 60-100 µL |
| Binding capacity | 60 µg |
| Time/ prep | 20 min/prep<br>(after lysis step) |
| Spin column | mini |

## 4. PREPARATION OF WORKING SOLUTIONS AND STORAGE CONDITIONS

***NOTE:*** ***Buffers BB3 and BBW*** **contain guanidine hydrochloride therefore wear gloves and goggles**.

All kit components can be stored at room temperature (18-25°C) through the expiration date printed on the packaging label.

Before starting any protocol with **SPEEDTOOLS TISSUE DNA EXTRACTION KIT** prepare the following reagents:

I. **Proteinase K:**

- ✓ **10 preps format:** Add **260 µL** of Proteinase Buffer to dissolve lyophilized Proteinase K.
- ✓ **50 preps format:** Add **1.35 mL** of Proteinase Buffer to dissolve lyophilized Proteinase K.

*The Proteinase K Solution is stable at –20°C for at least 6 months.*

**II. Buffer BB5:**

- ✓ **10 preps format:** Add **12 mL of molecular grade ethanol** (96-100%) to buffer BB5 concentrate. Mark the label of the bottle to indicate that ethanol was added.
- ✓ **50 preps format:** Add **48 mL of molecular grade ethanol** (96-100%) to buffer BB5 concentrate. Mark the label of the bottle to indicate that ethanol was added.

*Wash Buffer BB5 can be stored at room temperature (18-25°C) for at least 12 months.*

***Upon storage, especially at low temperatures, a white precipitate may form in Buffer BB3. Dissolve such precipitates by incubation of the bottle at 50-70°C before use.***

***NOTE:*** ***During storage, especially at low temperatures, a white precipitate may form in Buffers BT1 or Buffer BB3. Such precipitates can be easily dissolved by incubating the bottle at 50-70 °C before use.***

## 5. DNA ELUTION PROCEDURES

The *Standard Protocol* described in Section 6 has a recovery rate about 70-90%. It is possible to adapt the elution method and volume of elution buffer to the subsequent application of interest. In addition to the standard elution procedure there are several modifications possible. Use elution Buffer BBE 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 DNA will be about 80%.

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

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

Elution may also be performed with Tris-EDTA buffer (TE) of pH ≥ 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 BBE (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 BBE and storage, especially long term, at -20°C. Several freeze-thaw cycles will not interfere with most downstream applications. Possible exceptions are detection of trace amounts of DNA or long-range PCR (e.g. > 10kb). Multiple freeze-thaw cycles or prolonged storage of eluted DNA at 4°C or room temperature may influence detection sensitivities or reaction efficiencies due to DNA shearing or adsorption to surfaces.

# 6. INSTRUCTION FOR USE

## A. STANDARD PROTOCOL (for human or animal tissue and cultured cells)

**Before starting the preparation:**

- Check if Buffer BB5 and Proteinase K were prepared according to Section 4.
- Set an incubator or water bath to 56ºC and 70ºC.
- Before elution and if necessary preheat Elution Buffer BBE to 70ºC.

| STEP | DESCRIPTION | | |
|---|---|---|---|
| 1 | **PREPARE SAMPLE**<br>***Tissue***<br>Cut **25 mg** human or animal tissue into small pieces. Place the sample in a microcentrifuge tube. Proceed with Step 2.<br><br>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 centrifuge tube, add 50–75 µL phosphate buffered saline (PBS[1]) and homogenize.<br><br>***Cultured cells***<br>Resuspend up to **$10^7$ cells** in a final volume of **200 µL Buffer BT1**. Add **25 µL Proteinase K** solution and **200 µL Buffer BB3**. Vortex to mix and incubate the sample at **70°C** for 10-15 min. Proceed with Step 4. | | PREPARE SAMPLE |
| 2 | **PRE-LYSIS**<br>Add **180 µL Buffer BT1** and **25 µL Proteinase K** solution**.** Vortex to mix. Be sure that the samples are completely covered with lysis solution.<br>*If processing several samples, Proteinase K and Buffer BT1 may be premixed directly before use. Never mix Buffer BT1 and Proteinase K more than 10–15 min before addition to the sample: Proteinase K tends to self-digestion in Buffer BT1 without substrate.*<br>Incubate at **56°C** until complete lysis is obtained (at least **1–3 h**). Vortex occasionally during incubation or use a shaking incubator.<br>*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) and incubate for an additional 5 min at room temperature.* |  | +<br>180 µL<br>BUFFER BT1<br>+<br>25 µL<br>PROTEINASE K<br><br>Incubate<br>56ºC<br>1-3 h<br><br>Or<br><br>56ºC<br>overnight<br><br>Vortex |
| 3 | **SAMPLE LYSIS**<br>Vortex the samples. Add **200 µL** of lysis **Buffer BB3** to the samples and vortex the mixture vigorously. Incubate samples at **70°C** for 10 min. Vortex briefly.<br>*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.* |  | 200 µL<br>BUFFER BB3<br>+<br>Vortex<br>Incubate<br>70°C, 10 min |
| 4 | **ADJUST DNA BINDING CONDITIONS**<br>Add **210 µL** of **molecular grade ethanol** (96-100%) to each sample and vortex vigorously.<br>*After addition of ethanol a stringy precipitated 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.* |  | +<br>210 µL<br>MOLECULAR GRADE<br>ETHANOL<br>Vortex |

[1] Buffer PBS sterile: dissolve 8 g NaCl; 0.2 g KCl, 1.44 g $Na_2HPO_4$; 0.24 g $KH_2PO_4$ in 800 ml $H_2O$. Adjust pH to 7.4 with HCl. Add $H_2O$ to 1 Liter. Sterilise the Buffer PBS in the autoclave.

| | | | |
|---|---|---|---|
| 5 | **BIND DNA**<br>For each sample, place one column into a 2 ml collection tube and apply the sample to the column. Centrifuge at **11,000 × *g* for 1 min**. Discard the flow-through and place the column back into the collection tube.<br>*If the samples are not drawn completely through the matrix, repeat the centrifugation step at 11,000 × g. Discard flow-through.* |  | Load lysate into a column<br>1 min, 11,000 × *g* |
| 6 | **WASH SILICA MEMBRANE**<br>• ***Wash 1***<br>Add **500 µL Buffer BBW**. Centrifuge **1 min at 11,000 x *g***. Discard the flow-through and place the column into the collection tube.<br>• ***Wash 2***<br>Add **600 µL Buffer BB5** to the column. Centrifuge **1 min at 11,000 x g**. Discard the flow-through and place the column back into the collection tube. |  | +<br>500 µL<br>BUFFER BBW<br>1 min, 11,000 × *g*<br>+<br>600 µL<br>BUFFER BB5<br>1 min, 11,000 × *g* |
| 7 | **DRY SILICA MEMBRANE**<br>Place the column into 1.5 ml microcentrifuge tube and centrifuge **1 min at 11,000 x *g***.<br>*Residual ethanol is removed during this step.* |  | 1 min, 11,000 × *g* |
| 8 | **ELUTE HIGHLY PURE DNA**<br>Place the column into a new 1.5 ml microcentrifuge tube and add **100 µL Buffer BBE**. Dispense buffer directly onto the silica membrane. Incubate at **room temperature** for 1 min. Centrifuge **1 min at 11,000 x *g*.**<br>***For alternative DNA elution procedures see section 5***. |  | +<br>100 µL<br>BUFFER BBE<br>(70ºC)<br>Incubate RT<br>1 min<br>1 min,11,000 × *g* |

## B. Protocol for mouse or rat tails

**Before starting the preparation:**

- Check if Buffer BB5 and Proteinase K were prepared according to Section 4.
- Set an incubator or water bath to 56ºC.
- Before elution and if necessary preheat Elution Buffer BBE to 70ºC.

| STEP | DESCRIPTION | | |
|---|---|---|---|
| 1 | **PREPARE SAMPLE**<br>Cut **two 0.6 cm-pieces** of **mouse tail** or **one 0.6 cm-pieces** of **rat tail** and place them in a 1.5 ml tube. | | CUT 2 PIECES<br>(0.6 cm) |
| 2 | **PRE-LYSIS**<br>Add **180 µL Buffer BT1** and **25 µL Proteinase K.** Vortex to mix and incubate at **56ºC overnight** or until complete lysis is obtained.<br>*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).*<br>Vortex occasionally during incubation or use a shaking incubator. 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.<br>*If processing several samples, Proteinase K and Buffer BT1 may be premixed directly before use. Never mix Buffer BT1 and Proteinase K more than 10–15 min before addition to the sample: Proteinase K tends to self-digestion in Buffer BT1 without substrate.* |  | +<br>180 µL<br>BUFFER BT1<br>+<br>25 µL<br>PROTEINASE K<br><br>Incubate<br>56ºC, overnight<br>Vortex<br><br>Transfer 200 µL |
| 3 | **SAMPLE LYSIS**<br>Add **200 µL** of lysis **Buffer BB3** to the samples and vortex the mixture vigorously.<br>*Buffer BB3 and molecular grade ethanol can be premixed before addition to the lysate.* |  | +<br>200 µL<br>BUFFER BB3<br>Vortex |
| 4 | **Proceed with Step 4 of the *Standard Protocol*.** | | |

## C. Protocol for bacteria

**Before starting the preparation:**

- Check if Buffer BB5 and Proteinase K were prepared according to Section 4.
- Set an incubator or water bath to 56ºC and 70ºC.
- Before elution and if necessary preheat Elution Buffer BBE to 70ºC.

| STEP | DESCRIPTION | | |
|---|---|---|---|
| 1 | **PREPARE SAMPLE**<br>Centrifuge ≤ **1 ml culture** for **5 min at 8,000 x *g***. Remove supernatant.<br>*Depending on e.g. density of culture, culture medium, and bacterial strain, up to 1 ml of bacterial culture should be used.* | | BACTERIA CULTURE<br>(1 ml maximum) |
| 2 | **PRE-LYSIS**<br>Resuspend the pellet in **180 µL Buffer BT1** by pipetting up and down. Add **25 µL Proteinase K.** Vortex vigorously and incubate at **56ºC** until complete lysis is obtained (at least **1-3 h**). Vortex occasionally during incubation or use a shaking incubator. Samples can be incubated overnight.<br>*If RNA-free DNA is crucial for downstream applications, an RNase digestion may be performed: Add 20 µL RNase A (10 mg/ml) solution (not included) and incubate for an additional 5 min at room temperature.*<br>*Some strains, in particular Gram-positive bacteria, are more difficult to lyse. In such cases, a pre-incubation 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 BT1) supplemented with 20 mg/ml lysozyme or 0.2 mg/ml lysostaphin and incubate for 30-60 min at 37ºC.Add 25 µL Proteinase K, incubate at 56 °C until complete lysis is obtained.* |  | +<br>180 µL<br>BUFFER BT1<br>+<br>25 µL<br>PROTEINASE K<br>Vortex<br>Incubate 56ºC,<br>1-3 h/overnight |
| 3 | **Proceed with Step 3 of the *Standard Protocol*.** | | |

## D. Protocol for yeast

**Before starting the preparation:**

- Check if Buffer BB5 and Proteinase K were prepared according to Section 4.
- Check that 10 mM EDTA, pH 8.0 is available.
- Check that sorbitol buffer[2] and lyticase or zymolase[3] (not provided with the kit) is available for sample pre-lysis.
- Set an incubator or water bath to 30 ºC; 56ºC and 70ºC.
- Before elution and if necessary preheat Elution Buffer BBE to 70ºC.

| STEP | DESCRIPTION | | |
|---|---|---|---|
| 1 | **PREPARE SAMPLE**<br>Harvest **3 ml YPD yeast culture** ($OD_{600}$ ≤ 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**). |  | YEAST CULTURE<br>(3 ml YPD)<br><br>10 min, 5,000 × g<br><br>Wash the cells in 1 ml<br>10 mM EDTA pH 8 |
| 2 | **PRE LYSIS**<br>Resuspend the pellet in **600 µL sorbitol buffer**. Add **50 U lyticase** or **zymolase**. Incubate at **30ºC** for **30 min**. This step degrades the yeast cell wall creating spheroplasts (formation of spheroplast may be checked microscopically).<br><br>Centrifuge the mixture for **10 min** at **2,000 x *g***, remove supernatant and resuspend the pelleted spheroplasts in **180 µL Buffer BT1**. Add **25 µL Proteinase K** and vortex vigorously.<br><br>Incubate at **56ºC** 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.<br><br>*If RNA-free DNA is crucial for downstream applications, an RNase digestion may be performed: Add 20 µL RNase A (20 mg/ml) solution (not included) and incubate for an additional 5 min at room temperature.* |  <br>  | PELLET<br>600 µL sorbitol buffer<br>+<br>50 U lyticase/zymolase<br>Incubate 30ºC,<br>30 min<br><br>10 min, 2,000 × g<br>+<br>180 µL<br>BUFFER BT1<br>+<br>25 µL<br>PROTEINASE K<br><br>Incubate 56ºC,<br>1-3 h/overnight<br>Vortex |
| 3 | ***Proceed with Step 3 of the Standard Protocol.*** | | |

[2] Buffer sorbitol: 1.2 M sorbitol; 10 mM $CaCl_2$; 0.1 M Tris/HCl pH 7.5; 35 mM β-Mercaptoetanol.
[3] 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.

## E. Protocol for dried blood spots (e.g. Guthrie cards; NucleoCards; and FTA® cards)

**Before starting the preparation:**

- Check if Buffer BB5 and Proteinase K were prepared according to Section 4.
- Set an incubator or water bath to 94ºC and 56ºC.
- Before elution and if necessary preheat Elution Buffer BBE to 70ºC.

| .STEP | DESCRIPTION | | |
|---|---|---|---|
| 1 | **PREPARE SAMPLE**<br>Cut **one or two dried blood spots** as accurately as possible. Cut spots into small pieces and place them in a 1.5 ml centrifuge tube.<br>*The area of the dried blood spots should be between 15 and 30 $mm^2$.* | | SAMPLE |
| 2 | **PRE-LYSIS**<br>Add **180 µL Buffer BT1** and mix by vortexing. Spin the samples briefly and place them in a water bath or heating block and heat **10 min at 94ºC**.<br>Let the sample cool down. Add **25 µL Proteinase K**, vortex and incubate at **56ºC** for **1h**. Vortex occasionally during incubation or use a shaking water bath.<br>*Be sure that the samples are completely covered with lysis buffer during incubation.* |  | +<br>180 µL<br>BUFFER BT1<br>Incubate 94ºC, 10 min<br><br>Cool<br>+<br>25 µL<br>PROTEINASE K<br>Incubate 56ºC, 1 h<br>Vortex |
| 3 | **SAMPLE LYSIS**<br>Add **200 µL Buffer BB3** vortex vigorously and incubate at **56ºC** for **10 min**. | | 200 µL<br>BUFFER BB3<br>Vortex<br><br>Incubate 56ºC, 10 min |
| 4 | ***Proceed with Step 4 of the Standard Protocol.*** | | |

## F. Protocol for genomic DNA and viral DNA from blood samples

**Before starting the preparation:**

- Check if Buffer BB5 and Proteinase K were prepared according to Section 4.
- Set an incubator or water bath to 70ºC.
- Before elution and if necessary preheat Elution Buffer BBE to 70ºC.

| .STEP | DESCRIPTION | | |
|---|---|---|---|
| 1-2 | **PREPARE SAMPLE AND PRE-LYSIS**<br>These steps are not necessary for the present protocol. | | Not necessary |
| 3 | **SAMPLE LYSIS**<br>Add **25 µL Proteinase K** and up to **200 µL blood,** buffy coat, or body fluid sample (equilibrated to room temperature).<br>*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.*<br>Add **200 µL Buffer BB3** to the samples and vortex the mixture vigorously (10-20 sec).<br>Incubate samples at **room temperature** for **5 min.** Mix and incubate sample at **70 ºC** for **10-15 min.**<br>*The lysate should become brownish during incubation with Buffer BB3. Increase incubation time with Proteinase K (up to 30 min) and vortex one or twice vigorously during incubation if processing older or clotted blood samples.* |  | 25 µL<br>PROTEINASE K<br>+<br>200 µL sample<br>+<br>200 µL<br>BUFFER BB3<br>Incubate<br>RT, 5 min<br>Incubate<br>70ºC, 10-15 min |
| 4 | **ADJUST DNA BINDING CONDITIONS**<br>Add **210 µL molecular grade ethanol** (96-100 %), to each sample, and vortex again. |  | 210 µL<br>Molecular Grade Ethanol<br>Vortex |
| 5 | **BIND DNA**<br>For each sample, place one column into a 2 ml collection tube and apply the sample to the column. Centrifuge at **11,000 × *g* for 1 min**. Discard the flow-through and place the column back into the collection tube.<br>*If the samples are not drawn completely through the matrix, repeat the centrifugation step at 11,000 × g. Discard flow-through.* |  | Load lysate<br>into a column<br>1 min, 11,000 × g |
| 6 | ***Proceed with Step 4 of the Standard Protocol.*** | | |

## G. Protocol for hair roots

**Before starting the preparation:**

- Check if Buffer BB5 and Proteinase K were prepared according to Section 4.
- Set an incubator or water bath to 56ºC and 70ºC.
- Before elution and if necessary preheat Elution Buffer BBE to 70ºC.

| STEP | DESCRIPTION | | |
|---|---|---|---|
| 1 | **PREPARE SAMPLE**<br>Cut off the hair roots from the **hair sample** (up to 100) and collect them in 1.5 ml collection tube. | | HAIR ROOTS |
| 2 | **PRE-LYSIS**<br>Add **180 µL Buffer BT1** to the hair roots and freeze the samples in liquid nitrogen.<br>Thaw samples in a **56ºC** water bath. Repeat this procedure 4 times.<br>Add **25 µL Proteinase K**, mix by vortexing, and incubate **6-8 h** or overnight at **56ºC**. Use a shaking water bath/incubator or vortex occasionally. |  | +<br>180 µL<br>BUFFER BT1<br><br>5 cycles of freeze/thaw with liquid Nitrogen<br><br>25 µL<br>PROTEINASE K<br><br>Incubate 56ºC,<br>6-8 h/overnight<br>Vortex |
| 3 | ***Proceed with Step 3 of the Standard Protocol.*** | | |

## H. Protocol from paraffin-embedded tissue

**Before starting the preparation:**

- Check if Buffer BB5 and Proteinase K were prepared according to Section 4.
- Check that n-octane or xylene is available.
- Set an incubator or water bath to 37ºC; 56ºC; and 70ºC.
- Before elution and if necessary preheat Elution Buffer BBE to 70ºC.

| STEP | DESCRIPTION | | |
|---|---|---|---|
| 1 | **PREPARE SAMPLE**<br>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.<br>Add **1 ml n-octane** or **xylene** to each tube. Vortex vigorously and incubate at **room temperature** for about **30 min**. Vortex occasionally.<br>Centrifuge at **11,000 x *g*** for **3 min**. Pipette off supernatant.<br>Add **1 mL molecular grade 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.<br>Repeat the ethanol washing step. Pipette off as much of the ethanol as possible.<br>Incubate the open tube at **37ºC** until the ethanol has evaporated (~**15 min**). |  <br>  <br>  | SMALL SECTIONS<br>+<br>1 mL n-OCTANE<br><br>Vortex<br>Incubate RT, 30 min<br><br>3 min, 11,000 × g<br><br>+<br>1 mL ETANOL<br>3 min, 11,000 × g<br>+<br>1 mL MOLECULAR GRADE ETHANOL<br>3 min, 11,000 × g<br><br>Incubate open tube at 37ºC |
| 2 | ***Proceed with Step 2 of the Standard Protocol.*** | | |

## I. Protocol for detection of *Mycobacterium tuberculosis* or *Legionella pneumophila* in sputum or bronchoalveolar lavage

**Before starting the preparation:**

- Check if Buffer BB5 and Proteinase K were prepared according to Section 4.
- Prepare N-acetyl cystein/NaOH[4].
- Set an incubator or water bath to 56ºC and 70ºC.
- Before elution and if necessary preheat Elution Buffer BBE to 70ºC.

| STEP | DESCRIPTION | | |
|---|---|---|---|
| 1 | **PREPARE SAMPLE**<br>Add **200-500 µL sputum** or bronchoalveolar lavage to an **equal volume N-acetyl cystein/NaOH**. Vortex gently to mix.<br>Incubate the mixture for **25 min** at **room temperature** with shaking.<br>Adjust the volume to **25 mL** with **sterile water**. Centrifuge for **30 min** at **4,000 x *g***. Discard the supernatant.<br>Resuspend the pellet in **0.5-1 mL Buffer BT1**, depending on sample viscosity (Kit only includes 20 mL of Buffer BT1).<br>Transfer **200 µL** of the **resuspended sample** to a new microcentrifuge tube.<br>*For this protocol depending on the number and the viscosity of the samples you may require and additional bottle of Buffer BT1 (BIOTOOLS Ref. 21.161).* |  | ESPUTUM 200-500 µL<br>+<br>equal vol<br>N-acetyl cystein/NaOH<br>Vortex<br>Incubate<br>RT 25 min<br>Adjust vol to 25 mL<br>with sterile $H_2O$<br>30 min, 4,000 × g<br>Resuspend pellet<br>in 0.5-1 mL<br>BUFFER BT1<br>Transfer 200 µL |
| 2 | ***Proceed with Step 2 of the Standard Protocol.*** | | |

## J. Protocol for extraction of genomic DNA from stool

For this protocol depending on the number and the quality of the samples you may require and additional bottle of Buffer BT1 (BIOTOOLS Ref. 21.161).

**Before starting the preparation:**

- Check if Buffer BB5 and Proteinase K were prepared according to Section 4.
- Check if TE buffer[5] is available.
- Set an incubator or water bath to 56ºC and 70ºC.
- Before elution and if necessary preheat Elution Buffer BBE to 70ºC.

| STEP | DESCRIPTION | | |
|---|---|---|---|
| 1 | **PREPARE SAMPLE**<br>Add **250 mg feces** to **1 mL TE buffer.** Resuspend the sample by vigorously vortexing (**30 s**).<br>Centrifuge the sample for **15 min** at **4,000 x *g***. Discard the supernatant. Resuspend the pellet in **0.2-1 mL Buffer BT1**. Use as much buffer as necessary for good resuspension of the sample.<br>*The prepared pellet contains, among other constituents, cells from the digestive tract and bacteria.*<br>Transfer **200 µL** of the **resuspended sample** to a new microcentrifuge tube. |  | FECES (250 mg)<br>+<br>1 mL buffer TE<br>Vortex<br>15 min, 4,000 × g<br>Resuspend in<br>0.2-1 mL<br>BUFFER BT1<br>Transfer 200 µL |
| 2 | ***Proceed with the addition of 25 µL Proteinase K in Step 2 of the Standard Protocol.*** | | |

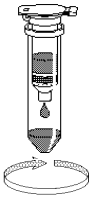
[4] N-acetil cystein/NaOH: 2 g NaOH; 1.45 g sodium citrate; 0.5 g N-acetyl cystein. Add water to 100 ml.
[5] Buffer TE: 10 mM Tris/HCl; 1 mM EDTA, pH 8.

## K. Protocol for viral DNA from stool

For this protocol you will require additional bottles of Proteinase K solution (BIOTOOLS Ref. 20050).

**Before starting the preparation:**

- Check if Buffer BB5 and Proteinase K were prepared according to Section 4.
- Prepare 0.9 % NaCl.
- Set an incubator or water bath to 70 ºC.
- Before elution and if necessary preheat Elution Buffer BBE to 70ºC.

| STEP | DESCRIPTION | | |
|---|---|---|---|
| 1 | **PREPARE SAMPLE**<br>Suspend the stool sample in **0.9 % NaCl.**<br>Centrifuge **aliquots** of the **stool sample** (1 mL) for **5 min** at **800** × ***g*** at **room temperature.**<br>Carefully reunite supernatant (do not touch the pellet).<br>Filtrate supernatant through 0.22-0.45 µm.<br>Fractionate the filtrate and centrifuge for **1 min** at **11,000** × ***g.*** |  | PREPARE SAMPLE<br>+<br>0.9% NaCl<br>1 min, 800 × *g*<br>Filtrate sample<br>1 min, 11,000 × *g* |
| 2 | **PRE-LYSIS**<br>Carefully remove the supernatant by decanting. Add **400 µL Buffer BT1** and **35 µL Proteinase K** solution**.** Vortex to mix. |  | +<br>400 µL<br>BUFFER BT1<br>+<br>35 µL<br>PROTEINASE K |
| 3 | **SAMPLE LYSIS**<br>Vortex the samples. Add **400 µL** of lysis **Buffer BB3** to the samples and vortex the mixture vigorously. Incubate samples at **70°C** for at least 30 min. Vortex briefly. |  | 400 µL<br>BUFFER BB3<br>+<br>Vortex<br>Incubate<br>70°C, 30 min |
| 4 | **ADJUST DNA BINDING CONDITIONS**<br>Add **420 µL** of **molecular grade ethanol** (96-100%) to each sample and vortex vigorously. |  | +<br>420 µL<br>MOLECULAR GRADE ETHANOL<br>Vortex |
| 5 | **BIND DNA**<br>For each sample, place one column into a 2 ml collection tube and apply the sample to the column. Centrifuge at **4,500** × ***g* for 1 min**. Discard the flow-through and place the column back into the collection tube.<br>*If the samples are not drawn completely through the matrix, repeat the centrifugation step at 11,000 × g. Discard flow-through.* |  | Load lysate into a column<br>1 min, 4,500 × *g* |
| 6 | **WASH SILICA MEMBRANE**<br>• ***Wash 1***<br>Add **600 µL Buffer BBW**. Centrifuge **1 min at 4,500 x *g***. Discard the flow-through and place the column into the collection tube.<br>• ***Wash 2***<br>Add **600 µL Buffer BB5** to the column. Centrifuge **1 min at 4,500 x g**. Discard the flow-through and place the column back into the collection tube.<br>• ***Wash 3***<br>Add **600 µL Buffer BB5** to the column. Centrifuge **2 min at 11,000 x g**. Discard the flow-through and place the column back into the collection tube. |  | +<br>600 µL<br>BUFFER BBW<br>1 min, 4,500 × *g*<br>+<br>600 µL<br>BUFFER BB5<br>1 min, 4,500 × *g*<br>+<br>600 µL<br>BUFFER BB5<br>2 min, 11,000 × *g* |

| | | | |
|---|---|---|---|
| 7 | **DRY SILICA MEMBRANE**<br>Place the column into 1.5 ml microcentrifuge tube and indubate with open lid for **1-2 min** at **70 ºC.**<br>*Residual ethanol is removed during this step.* |  | Incubate<br>70°C, 1-2 min |
| 8 | **ELUTE HIGHLY PURE DNA**<br>Place the column into a new 1.5 ml microcentrifuge tube and add **100 µL Buffer BBE**. Dispense buffer directly onto the silica membrane. Incubate with closed lid for **3-5 min.**<br>Centrifuge **1 min at 4,500 x *g*.**<br>***For alternative DNA elution procedures see section 5***. |  | +<br>100 µL<br>BUFFER BBE<br>Incubate RT<br>3-5 min<br>1 min 4,500 × *g* |

## L. Protocol for detection of EHEC bacteria in food (e.g. fresh cows' milk)

In humans, Vero toxin-forming *E. coli* strains (VTEC, EHEC) can cause diseases. The main reservoirs and sources of infection for humans are horned cattle and the corresponding foods, especially raw or insufficiently cooked minced meat and raw milk.

**Before starting the preparation:**

- Check if Buffer BB5 and Proteinase K were prepared according to Section 4.
- Prepare mTSB[6] and 3.2 M sodium acetate.
- Check if Novobiocin is available.
- Set an incubator or water bath to 37 ºC; 56ºC and 70ºC.
- Before elution and if necessary preheat Elution Buffer BBE to 70ºC.

| STEP | DESCRIPTION | | |
|---|---|---|---|
| 1 | **PREPARE SAMPLE**<br>To a sterile 1 liter flask, add **25 mL milk** and **225 mL pre-warmed** (37ºC) **mTSB medium** (supplied with Novobiocin). Incubate the mixture in a shaking water bath for **5-6 h** or overnight at **37ºC**.<br>Centrifuge **100 mL culture** for **40 min** at **6,000 x *g***.<br>Gently pour off the supernatant and resuspend the pellet in 2 mL sterile water.<br>Centrifuge for **10 min** at **10,000 x g**. |  | MILK (25 mL)<br>+<br>225 mL sterile mTSB<br>Incubate 37ºC with shaking,<br>5-6 h/overnight<br>100 mL culture<br>40 min, 6,000 × g<br>Resuspend cells<br>in 2 mL sterile $H_2O$<br>10 min, 10,000 × g |
| 2 | **PRE-LYSIS**<br>Resuspend the pellet in **180 µL Buffer BT1** and add **25 µL Proteinase K**. |  | +<br>180 µL<br>BUFFER BT1<br>+<br>25 µL<br>PROTEINASE K |
| 3-8 | ***Proceed with Step 3 of the Standard Protocol.***<br>***Perform two elution steps with the indicated elution volume of the Standard Protocol.*** | | 200 µL ELUATE |
| 9 | After elution of the DNA, proceed with the following step.<br>Precipitate the obtained DNA by adding **20 µL** of **3.2 M sodium acetate** and **400 µL of molecular grade ethanol** to 200 µL the eluate.<br>Centrifuge for **30 min** at **11,000 x *g***. Discard supernatant and wash the pellet with **1 mL 70% molecular grade ethanol** and resuspend in **10 µL sterile water**. |  | +<br>20 µL 3.2 M SODIUM ACETATE<br>+<br>400 µL MOLECULAR GRADE ETHANOL<br>30 min, 11,000 × g<br>Wash pellet with<br>1 mL MOLECULAR GRADE ETHANOL 70%<br>Resuspend in<br>10 µL sterile $H_2O$ |

[6] mTSB Medium: 30 g Tryptic soy broth (Gibco); 1.5 g biles salts No. 3 (Oxoid); 1.5 g $KH_2PO_4$. Add 900 ml de $H_2O$. 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 121ºC.

## M. Protocol for purification of bacterial DNA (e.g. *Borrelia burgdorferi*) from urine

**Before starting the preparation:**

- Check if Buffer BB5 and Proteinase K were prepared according to Section 4.
- Set an incubator or water bath to 56ºC and 70ºC.
- Before elution and if necessary preheat Elution Buffer BBE to 70ºC.

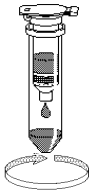
| STEP | DESCRIPTION | | |
|---|---|---|---|
| 1 | **PREPARE SAMPLE**<br>Centrifuge **1 mL urine** sample at **13,000 x g** for **30 min**. Discard the supernatant, add again **1 mL urine** sample to the pellet and centrifuge at **13,000 x *g*** for **30 min**. Repeat this step a third time.<br>*The sample material should be fresh and storage at –20 to –80ºC is only recommendable for a couple of days. After thawing incubate the sample at 40ºC as long as all precipitates, are dissolved. Urine tends to form precipitates when stored at low temperatures. If a complete solution does not happen let the precipitate sediment and proceed with step 1 of the protocol using only the supernatant* |  | 1 mL URINE<br>30 min, 13,000 × g<br>+<br>1 mL URINE<br>30 min, 13,000 × g<br>+<br>1 mL URINE<br>30 min, 13,000 × g |
| 2 | ***Proceed with the Step 2 of the Standard Protocol.*** | | |

## N. Protocol for purification of viral DNA (e.g. CMV) from urine

**Before starting the preparation:**

- Check if Buffer BB5 and Proteinase K were prepared according to Section 4.
- Set an incubator or water bath to 70ºC.
- Before elution and if necessary preheat Elution Buffer BBE to 70ºC.

| STEP | DESCRIPTION | | |
|---|---|---|---|
| 1 | **PREPARE SAMPLE**<br>Prepare **3-4 aliquots** (1 mL each aliquot) of **urine** sample. Centrifuge aliquots for **10 min** at **13,000 x *g*.** Carefully decant the supernatant of the aliquots.<br>If frozen urine samples are used precipitates may appear after defrosting, which must be dissolved before the centrifugation step. This can be done through a 30 min incubation step at 37-40ºC. If a complete solution does not happen let the precipitate sediment and proceed with Step 1 of the protocol using only the supernatant. |  | SAMPLE divided in aliquots of 1 mL<br>1 mL URINE<br>10 min, 13,000 × g |
| 2 | **PRE-LYSIS**<br>Resuspend the pellet in 180 µL Buffer BT1 and 25 µL Proteinase K.<br>Resuspend the first pellet with reagents indicated above. 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. |  | +<br>180 µL<br>BUFFER BT1<br>+<br>25 µL<br>PROTEINASE K |
| 3 | **SAMPLE LYSIS**<br>Add **200 µL** of lysis **Buffer BB3** and incubate at least **20 min** at **70°C**. Vortex briefly. |  | 200 µL<br>BUFFER BB3<br>Vortex and Incubate<br>70°C, 20 min |

| | | | |
|---|---|---|---|
| 4 | **ADJUST DNA BINDING CONDITIONS**<br>Add **210 µL** of **molecular grade ethanol** (96-100%) to the sample and vortex vigorously. |  | +<br>210 µL<br>MOLECULAR GRADE<br>ETHANOL<br>Vortex |
| 5 | **BIND DNA**<br>For each sample, place one column into a 2 mL collection tube and apply the sample to the column. Centrifuge at **4,500 × *g*** during **1 min**. Discard the flow-through and place the column back into the collection tube. |  | Load lysate<br>into a column<br><br>1 min, 4,500 × *g* |
| 6 | **WASH SILICA MEMBRANE**<br>• ***1st Wash***<br>Add **500 µL Buffer BBW**. Centrifuge **1 min** at **4,500 x *g***. Discard flow-through and place the column back into the collection tube.<br>• ***2nd Wash***<br>Add **600 µL Buffer BB5**. Centrifuge **2 min** at **11,000 x g**. Discard the flow-through and place the column back into the collection tube. |  | +<br>500 µL<br>BUFFER BBW<br>1 min, 4,500 × *g*<br><br>+<br>600 µL<br>BUFFER BBW<br>2 min, 11,000 × *g* |
| 7 | **DRY SILICA MEMBRANE**<br>Incubate with open lid for **1-2 min at 70ºC**.<br>*Residual ethanol is removed during this step.* | | *Incubate tubes*<br>*with open lid*<br>*Incubate 70º*<br>*1-2 min* |
| 8 | **ELUTE HIGHLY PURE DNA**<br>Add **70 µL Buffer BBE,** close the lid and incubate for further **3-5 min**.<br>Centrifuge **1 min** at **4,500 x *g***. |  | +<br>70 µL<br>BUFFER BBE<br><br>Incubate 70ºC<br>3-5 min<br>*1 min, 4,500 × g* |

## O. Protocol for purification of bacterial DNA (e.g. *Chamydia trachomatis*) from cultures, biological fluids or clinical specimens

**Before starting the preparation:**

- Check if Buffer BB5 and Proteinase K were prepared according to Section 4.
- Set an incubator or water bath to 56ºC and 70ºC.
- Before elution and if necessary preheat Elution Buffer BBE to 70ºC.

| STEP | DESCRIPTION | | |
|---|---|---|---|
| 1 | **PREPARE SAMPLE**<br>• Isolation of bacterial DNA from **bacterial cultures** or **biological fluids**: pellet bacteria by centrifugation for **5 min** at **13,000 x *g***.<br>• Isolation of bacterial DNA from **eye**, **nasal** or **pharyngeal swabs**: collect samples, add **2 mL PBS**[7] containing a common fungicide, and incubate for **several hours** at **room temperature**. Pellet bacteria by centrifugation for **5 min** at **13,000 x *g*** |  | SAMPLE<br><br>5 min, 13,000 × g |
| 2 | ***Proceed with Step 2 of the Standard Protocol.*** | | |

[7] Buffer PBS sterile: dissolve 8 g NaCl; 0.2 g KCl, 1.44 g $Na_2HPO_4$; 0.24 g $KH_2PO_4$ in 800 ml $H_2O$. Adjust pH to 7.4 with HCl. Add $H_2O$ to 1 Liter. Sterilise the Buffer PBS in the autoclave.

## P. Protocol for purification of genomic DNA from insects

**Before starting the preparation:**

- Check if Buffer BB5 and Proteinase K were prepared according to Section 4.
- Set an incubator or water bath to 56ºC and 70ºC.
- Before elution and if necessary preheat Elution Buffer BBE to 70ºC.

| STEP | DESCRIPTION | | |
|---|---|---|---|
| 1 | **PREPARE SAMPLE**<br>Homogenize not more than **50 mg insects** under liquid nitrogen and transfer the powder into a 1.5 mL centrifuge tube. | | SAMPLE<br>(< 50 mg)<br><br>Homogenize<br>under liquid nitrogen |
| 2 | ***Proceed with the Step 2 of the Standard Protocol.*** | | |

## Q. Protocol for purification of genomic DNA from dental swabs

**Before starting the preparation:**

- Check if Buffer BB5 and Proteinase K were prepared according to Section 4.
- Set an incubator or water bath to 70 ºC and 95 ºC.
- Before elution and if necessary preheat Elution Buffer BBE to 70ºC.

| STEP | DESCRIPTION | | |
|---|---|---|---|
| 1 | **PREPARE SAMPLE**<br>Place **swab material** (paper, cotton, brushes, plastic) in a 1.5 mL microcentrifuge tube. | | SAMPLE |
| 2 | **PRE-LYSIS**<br>Add **180 µL Buffer BT1** 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.<br><br>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***.<br><br>Incubate the tubes at **70ºC** 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 **95ºC** for **5 min**.<br><br>Spin briefly for **15 s** at **1,500 x g** to collect any sample from the lids. Open the microcentrifuge tubes.<br><br>*Depending on the bacterial strains that are to be detected, incubation at 95ºC can be skipped.* |  | 180 µL<br>BUFFER BT1<br>+<br>25 µL<br>PROTEINASE K<br><br>Spin<br>Incubate 5 min a TA<br>vortex and spin<br><br>Incubate<br>10 min, 70ºC<br><br>Incubate<br>5 min, 95ºC<br>Spin |
| 3 | **SAMPLE LYSIS**<br>Transfer as much as possible of the lysate solution to a 1.5 mL microcentrifuge tube. Discard swab and continue with recovered solution. | | |
| | ***Proceed with Step 3 of the Standard Protocol.*** | | |

## R. Protocol for purification of genomic DNA from buccal swabs

**Before starting the preparation:**

- Check if Buffer BB5 and Proteinase K were prepared according to Section 4.
- Check if PBS[8] is available.
- Set an incubator or water bath to 56ºC and 70ºC.
- Before elution and if necessary preheat Elution Buffer BBE to 70ºC.

| STEP | DESCRIPTION | | |
|---|---|---|---|
| 1 | **PREPARE SAMPLE**<br>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.<br>*The individual should not have consumed food or drink within 30 min before collection of the sample.* | | SAMPLE |
| 2 | **PRE-LYSIS**<br>Place the dry swab material in 2 mL microcentrifuge tube. Add **400-600 µL PBS** and **25 µL Proteinase K** to the swabs.<br>*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.*<br>Mix by vortexing **2 x 5 sec** and incubate **10 min** at **56ºC.** |  | +<br>400-600 µL<br>buffer PBS<br>+<br>25 µL<br>PROTEINASE K<br><br>Incubate<br>10 min, 56ºC |
| 3 | **SAMPLE LYSIS**<br>Transfer as much as possible of the lysate solution to a 1.5mL microcentrifuge tube.<br>Discard swab and continue with recovered solution.<br>Add **one volume Buffer BB3** (400-600 µL, depending on the swab type/volume of PBS buffer used) and vortex vigorously. Incubate the samples at **70ºC** for **10 min**. | | 1 vol<br>BUFFER BB3<br><br>Incubate<br>10 min, 70ºC |
| 4 | **ADJUST DNA BINDING CONDITIONS**<br>Add **one volume** (200-300 µL, depending on the swab type) **molecular grade ethanol** (96-100%) to each sample and mix by vortexing. |  | +<br>1 vol<br>MOLECULAR GRADE<br>ETHANOL<br>Vortex |
| 5 | **BIND DNA**<br>Transfer **600 µL** of the **samples** from the 2 mL microcentrifuge tubes into the columns. Centrifuge at **11,000 x g** for **1 min**.<br>*If the samples are not drawn through completely, repeat the centrifugation.*<br>Discard the flow-through and place the column back into the collection tube. Repeat Step 5 once or twice, depending on the lysis volume. |  | Load lysate<br>into a column<br><br>1 min, 11,000 × *g* |
| 6 | When all of the lysate has been applied to the columns, discard Collection Tube and place the column in a new Collection Tube.<br>***Proceed with Step 6 of the Standard Protocol.*** | | |

[8] Buffer PBS sterile: dissolve 8 g NaCl; 0.2 g KCl, 1.44 g $Na_2HPO_4$; 0.24 g $KH_2PO_4$ in 800 ml $H_2O$. Adjust pH to 7.4 with HCl. Add $H_2O$ to 1 Liter. Sterilise the Buffer PBS in the autoclave.

## 7. TROUBLESHOOTING

| Problem | Possible cause and suggestions |
|---|---|
| No yield or poor DNA yield | ***Incomplete cell lysis***<br>• Sample not thoroughly homogenized and mixed with Buffer BT1 / Proteinase K. The mixture has to be vortexed vigorously immediately after addition of Buffer BT1.<br>• Decreased Proteinase K activity: store dissolved Proteinase K at –20ºC for at least 6 months.<br>***Reagents not applied properly***<br>• Prepare Buffers BB3, BB5, and Proteinase K according to instructions (section 4). Add molecular grade ethanol to lysates before loading them on the columns.<br>***Suboptimal elution of DNA from the column***<br>• Preheat Buffer BBE to 70°C before elution. Apply Buffer BBE directly onto the center of the silica membrane.<br>• Elution efficiencies decrease dramatically if elution is done with buffers with pH < 7.0. Use slightly alkaline elution buffers like Buffer BBE (pH 8.5).<br>• Especially when expecting high yields from large amounts of material, we recommend elution with 200 µL Buffer BBE and incubation of the closed columns in an incubator at 70ºC for 5 min before centrifugation. |
| Poor DNA quality | ***Incomplete lysis***<br>• Sample not thoroughly homogenized and mixed with Buffer BT1 / Proteinase K. The mixture has to be vortexed vigorously immediately after addition of Buffer BT1.<br>• Decreased Proteinase K activity: store dissolved Proteinase K at –20ºC for at least 6 months.<br>***Reagents not applied properly***<br>• Prepare Buffers BB3, BB5, and Proteinase K according to instructions (section 4). Add molecular grade ethanol to lysates before loading them on the columns.<br>***RNA in sample***<br>• If DNA free of RNA is desired, add RNase A solution, not supplied with the kit, before addition of lysis Buffer BB3 and incubate at 37ºC for 5 min. |
| Columns clogged | ***Too much sample material used***<br>• 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 BB3 and molecular grade ethanol.<br>***Incomplete lysis***<br>• Sample not thoroughly homogenized and mixed with Buffer BT1 / Proteinase K. The mixture has to be vortexed vigorously immediately after addition of Buffer BT1.<br>• Decreased Proteinase K activity: store dissolved Proteinase K at –20ºC for at least 6 months.<br>***Reagents not applied properly***<br>• Prepare Buffer BB5 and Proteinase K according to instructions (section 4). Add molecular grade ethanol to lysates before loading them on the columns. |

| | |
|---|---|
| Suboptimal performance of genomic DNA in enzymatic reactions | ***Carryover of ethanol or salt***<br>• Be certain to centrifuge ≥ 1 min at 11,000 x *g* in order to remove all of ethanolic Buffer BB5 before eluting the DNA. If, for any reason, the level of Buffer BB5 has reached the column outlet after drying, repeat the centrifugation.<br>• Do not chill Buffer BB5 before use. Cold buffer will not remove salt effectively. Equilibrate Buffer BB5 to room temperature before use.<br>***Contamination of DNA with inhibitory substances***<br>• DNA eluated with Tris/EDTA buffer (TE). EDTA may inhibit enzymatic reactions. Repurify DNA and eluate in Buffer BBE.<br>• If the A260/280 ratio of the eluate is □ 1.6, repeat the purification procedure: add 1 vol Buffer BB3 plus 1 vol molecular grade ethanol (96-100%) to the eluate. Load the mixture onto a column and proceed with step 5 of the Standard Protocol. |

## 8. ORDERING INFORMATION

| **SPEEDTOOLS KIT** | **10 PREPS** | **50 PREPS** | **250 PREPS** |
|---|---|---|---|
| SPEEDTOOLS **DNA EXTRACTION** KIT | Ref. 21.130M | Ref. 21.131 | Ref. 21.132 |
| SPEEDTOOLS **TISSUE DNA EXTRACTION** KIT | Ref. 21.135M | Ref. 21.136 | Ref. 21.137 |
| SPEEDTOOLS **RNA VIRUS EXTRACTION** KIT | Ref. 21.140M | Ref. 21.141 | Ref. 21.142 |
| SPEEDTOOLS **FOOD DNA EXTRACTION** KIT | Ref. 21.175M | Ref. 21.176 | Ref. 21.177 |
| SPEEDTOOLS **PLANT DNA EXTRACTION** KIT | Ref. 21.170M | Ref. 21.171 | Ref. 21.172 |
| SPEEDTOOLS **TOTAL RNA EXTRACTION** KIT | Ref. 21.210M | Ref. 21.211 | Ref. 21.212 |
| SPEEDTOOLS **PCR CLEAN-UP** KIT | Ref. 21.200M | Ref. 21.201 | Ref. 21.202 |
| SPEEDTOOLS **PLASMID DNA PURIFICATION** KIT | Ref. 21.220M | Ref. 21.221 | Ref. 21.222 |

# 9. PRODUCT USE RESTRICTION AND WARRANTY

1. Product for research purposes and *in vitro uses* only.
2. No claim or representation is intended for its use to identify any specific microorganism or for clinical use (diagnostic, prognosis, therapeutic, or blood bank).
3. It is rather the responsibility of the user to verify the use of the kit for a specific application range as the performance characteristic of this kit has not been verified for a specific microorganism.
4. The kit includes documentation stating specifications and other technical information. Follow the instructions in order to obtain highly pure genomic DNA.
5. BIOTOOLS warrants to meet the stated specifications of the kit. Any product not fulfilling the specifications included in the product sheet will be replaced.
6. BIOTOOLS obligation and purchaser's rights under this warranty are limited to the replacement by BIOTOOLS of any product that is shown defective in fabrication, and that must be returned to BIOTOOLS, freight prepaid, or at BIOTOOLS' option, replacement of the purchasing price.
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