



BIOTOOLS

BIOTOOLS B&M LABS.S.A.

Manufactured by:

BIOTOOLS B&M Labs, S.A.
Valle de Tobalina - 52 - Nave 39
28021 Madrid
Spain

Tel. (34) 91 710 00 74
Fax (34) 91 505 31 18
e-mail: info@biotools.eu
www.biotools.eu

SPEEDTOOLS TISSUE DNA EXTRACTION KIT

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

Instructions for Use (Cat. No. 21.136/7)

**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	2
2. KIT CONTENTS	2
3. INTENDED USE	2
4. PREPARATION OF WORKING SOLUTIONS AND STORAGE CONDITIONS	3
5. DNA ELUTION PROCEDURES	3
6. SAFETY INSTRUCTIONS	4
7. INSTRUCTION FOR USE	5
A. STANDARD PROTOCOL (for human or animal tissue and cultured cells).....	5
B. Protocol for mouse or rat tails.....	6
C. Protocol for bacteria.....	7
D. Protocol for purification of bacterial DNA e.g. <i>Chlamydia trachomatis</i> from cultures, biological fluids or clinical specimens	7
E. Protocol for yeast	8
F. Protocol for dried blood spots (Guthrie cards).....	8
G. Protocol for hair roots.....	9
H. Protocol from paraffin-embedded tissue	9
I. Protocol for detection of <i>Mycobacterium tuberculosis</i> or <i>Legionella pneumophila</i> in sputum or bronchoalveolar lavage.....	10
J. Protocol for extraction of genomic DNA from feces.....	10
K. Protocol for detection of EHEC bacteria in food (e.g. fresh cows' milk).....	11
L. Protocol for purification of bacterial DNA e.g. <i>Borrelia burgdorferi</i> from urine.....	12
M. Protocol for purification of viral DNA e.g. cytomegalovirus from urine	12
N. Protocol for purification of genomic DNA from insects	13
O. Protocol for purification of genomic DNA from dental swabs.....	14
P. Protocol for purification of genomic DNA from buccal swabs	14
8. TROUBLESHOOTING	15
9. ORDERING INFORMATION.....	17
10.PRODUCT USE RESTRICTION AND WARRANTY.....	17

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 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	50 Preps Cat. No. 21.136	250 Preps Cat. No. 21.137
Buffer BT1	20 ml	5 x 20 ml
Buffer BB1	10 ml	5 x 10 ml
Reagent BB2	2.5 ml	5 x 2.5 ml
Buffer BB5 (concentrate)	7 ml	5 x 7 ml
Buffer BBW	30 ml	5 x 30 ml
Buffer BBE	13 ml	5 x 13 ml
Proteinase K (lyophilized)	30 mg	5 x 30 mg
Proteinase Buffer	1.8 ml	5 x 1.8 ml
Speedtools Tissue Columns	50	5 x 50
2 ml Collecting Tubes	100	5 x 100
Label for Buffer BB3	1	5 x 1
Protocol	1	5 x 1

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	
SAMPLE SIZE	25 mg / 10 ⁷ cells
AVERAGE YIELD	35 µg
ELUTION VOLUME	100 µl
BINDING CAPACITY	60 µg
TIME / PREP	20 min / 4-6 preps (after lysis of sample)
SPIN COLUMN TYPE	mini

4. PREPARATION OF WORKING SOLUTIONS AND STORAGE CONDITIONS

NOTE

Buffers BB1, BB3 and BBW contain guanidine hydrochloride therefore wear gloves and goggles.

All kit components can be stored at room temperature (20-25°C) and are stable up to one year.

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

- **Proteinase K:** Add 1.35 ml of Proteinase Buffer to dissolve lyophilized Proteinase K. This solution is stable at +4°C for up to 6 months. Storage at –20°C is recommended if the solution will not be used up during this period.
- **Buffer BB3:** Transfer Buffer BB1 to Reagent BB2 completely and mix well. The resulting solution (a label is provided) Buffer BB3 is stable for up to one year at room temperature (20-25°C).
- **Buffer BB5:** Add 28 ml of ethanol (96-100%) to buffer BB5 concentrate. Store Buffer BB5 at room temperature (20-25°C) for up to one year.

Upon storage, especially at low temperatures, a white precipitate may form in Buffers BT1, BB1 or Buffer BB3. Dissolve such precipitates by incubation of 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 Protocol* there are several modifications possible. Use elution Buffer BBE preheated to 70°C 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 *Standard Protocol*. Concentration of DNA will be ca. 30% higher than with standard elution. Maximal yield of bound nucleic acid is about 80%.
- High yield and high concentration:** Apply half the volume of elution buffer indicated in the *Standard 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 (100 µl) at a high concentration.

NOTE

For convenience, elution buffer of ambient temperature may be used. This will result in a somewhat lower yield (approximately 20%) compared to elution with heated elution buffer.

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 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 elution 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.

Performance of long-range PCR (e.g. > 10kb) or detection sensitivity of trace amount of DNA species might be reduced after multiple freeze-thaw cycles, or prolonged storage of eluted DNA at 4°C or room temperature due to shearing of DNA or adsorption to surfaces.

6. SAFETY INSTRUCTIONS

The following components of the SPEEDTOOLS TISSUE DNA EXTRACTION KIT contain hazardous contents. ***Wear gloves and goggles and follow the safety instructions given in this section.***




Reagent	Hazard Contents	Hazard Symbol	Hazard Symbol	Risk Phrases	Safety Phrases
BB1	Guanidine hydrochloride	Xⁿ	Xn*	<u>Harmful if swallowed</u> <u>Irritating to eyes and skin</u> <u>Do not breathe dust</u>	R 22-36/38 S 22
BBW	Guanidine hydrochloride	Xⁿ	Xn*	<u>Harmful if swallowed</u> <u>Irritating to eyes and skin</u> <u>Do not breathe dust</u>	R 22-36/38 S 22
Proteinase K	Proteinase K, lyophilised	Xⁿ	Xn*	<u>Irritating to eyes, respiratory system and skin</u> <u>May cause sensitisation by inhalation</u> <u>Do not breathe dust</u> <u>Avoid contact with the skin</u> <u>In case of contact with eyes, rinse immediately with plenty of water and seek medical advice</u> <u>Wear suitable protective clothing and gloves</u>	R 36/37/38-42 S 22-24-26-36/37

* Label not necessary, if quantity below 125 g or ml (concerning 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 42 and TRGS 200 7.1)

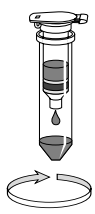
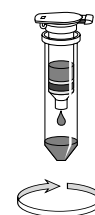

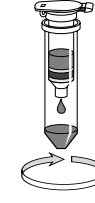
7. INSTRUCTION FOR USE

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

Before starting with the preparation, set incubators or water baths to 56°C and 70°C, respectively. Equilibrate elution Buffer BBE to 70°C.


STEP	DESCRIPTION		
1	<p>PREPARE SAMPLE</p> <p>Tissue Cut 25 mg human or animal tissue into small pieces. Place the sample in a microcentrifuge tube. Proceed with Step 2.</p> <p>Cultured cells Resuspend up to 10⁷ cells in a final volume of 200 µl Buffer BT1. Add 25 µl Proteinase K solution and 200 µl Buffer BB3. Incubate the sample at 70°C for 10-15 min. Proceed with Step 4.</p> <p><i>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 (PBS¹) and homogenize.</i></p>		PREPARE SAMPLE
2	<p>PRE-LYSIS</p> <p>Add 180 µl Buffer BT1 and 25 µl Proteinase K. Vortex to mix. Be sure that the samples are completely covered with lysis solution.</p> <p><i>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.</i></p> <p>Incubate at 56°C until complete lysis is obtained (at least 1–3 h). Vortex occasionally during incubation or use a shaking incubator.</p> <p><i>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 (20 mg/ml) solution (not included) and incubate for an additional 5 min at room temperature.</i></p>		<p>+ 180 µl BUFFER BT1 + 25 µl PROTEINASE K</p> <p>Incubate 56°C 1-3 h/overnight</p> <p>Vortex</p>
3	<p>SAMPLE LYSIS</p> <p>Vortex the samples. Add 200 µl lysis Buffer BB3 to the samples and vortex the mixture vigorously. Incubate samples at 70°C for 10 min. Vortex briefly.</p> <p><i>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.</i></p> <p><i>Buffer BB3 and ethanol can be premixed before addition of the lysate</i></p>		<p>200 µl BUFFER BB3 + Vortex Incubate 70°C, 10 min</p>
4	<p>ADJUST DNA BINDING CONDITIONS</p> <p>Add 210 µl ethanol (96-100%) to each sample and vortex vigorously.</p> <p><i>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.</i></p>		<p>+ 210 µl ETHANOL Vortex</p>

¹ Buffer PBS sterile: dissolve 8 g NaCl; 0.2 g KCl, 1.44 g Na₂HPO₄; 0.24 g KH₂PO₄ in 800 ml H₂O. Adjust pH to 7.4 with HCl. Add H₂O to 1 Liter. Sterilise the Buffer PBS in the autoclave.

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


B. Protocol for mouse or rat tails

Before starting with the preparation, set incubators or water baths to 56°C and 70°C, respectively. Equilibrate elution Buffer BBE to 70°C.

STEP	DESCRIPTION		
1	<p>PREPARE SAMPLE</p> <p>Cut two 0.6 cm-pieces of mouse tail and place them in a 1.5 ml centrifuge tube. If processing rat tails, one 0.6 cm-piece is sufficient.</p>		<p>CUT 2 PIECES (0,6 cm)</p>
2	<p>PRE-LYSIS</p> <p>Add 180 µl Buffer BT1 and 25 µl Proteinase K. Vortex to mix and incubate at 56°C overnight or until complete lysis is obtained. 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 × g). Transfer 200 µl supernatant to a new tube.</p> <p><i>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.</i></p>		<p>+ 180 µl BUFFER BT1 + 25 µl PROTEINASE K</p> <p>Incubate 56°C, overnight Vortex</p> <p>Transfer 200 µl</p>
3	<p>SAMPLE LYSIS</p> <p>Add 200 µl lysis Buffer BB3 to the samples and vortex the mixture vigorously.</p>		<p>+ 200 µl BUFFER BB3 Vortex</p>
4	<p>Proceed with Step 4 of the <i>Standard Protocol</i>.</p>		


C. Protocol for bacteria

Before starting with the preparation, set incubators or water baths to 56°C and 70°C, respectively. Equilibrate elution Buffer BBE to 70°C.

STEP	DESCRIPTION		
1	<p>PREPARE SAMPLE</p> <p>Depending on e.g. density of culture, culture medium, and bacterial strain, up to 1 ml of bacterial culture should be used.</p> <p>Centrifuge up to 1 ml culture for 5 min at 8,000 x g. Remove supernatant.</p>		BACTERIA CULTURE (1 ml maximum)
2	<p>PRE-LYSIS</p> <p>Resuspend the pellet in 180 µl Buffer BT1 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.</p> <p><i>If RNA-free DNA is crucial for downstream applications, a RNase digest may be performed: Add 20 µl RNase A (20 mg/ml) solution (not included) and incubate for an additional 5 min at room temperature.</i></p> <p><i>Some strains, specially 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 BT1) supplemented with 20 mg/ml lysozyme or 0.2 mg/ml lysostaphin and incubate for 30-60 min at 37°C.</i></p>		<p>+ 180 µl BUFFER BT1 + 25 µl PROTEINASE K</p> <p>Vortex Incubate 56°C, 1-3 h/overnight</p>
3	Proceed with Step 3 of the <i>Standard Protocol</i> .		

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




Before starting with the preparation, set incubators or water baths to 56°C and 70°C, respectively. Equilibrate elution Buffer BBE to 70°C.

STEP	DESCRIPTION		
1	<p>PREPARE SAMPLE</p> <ul style="list-style-type: none"> 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. Isolation of bacterial DNA from eye, nasal or pharyngeal swabs: collect samples, add 2 ml PBS² 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 5 min, 13,000 x g
2	Proceed with Step 2 of the <i>Standard Protocol</i> .		

² Buffer PBS sterile: dissolve 8 g NaCl; 0.2 g KCl, 1.44 g Na₂HPO₄; 0.24 g KH₂PO₄ in 800 ml H₂O. Adjust pH to 7.4 with HCl. Add H₂O to 1 Liter. Sterilise the Buffer PBS in the autoclave.


E. Protocol for yeast

Before starting with the preparation, set incubators or water baths to 30°C, 56°C and 70°C, respectively. Equilibrate elution Buffer BBE to 70°C.

STEP	DESCRIPTION		
1	PREPARE SAMPLE Harvest 3 ml YPD yeast culture (OD ₆₀₀ up to 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 (3 ml YPD) 10 min, 5,000 x g Wash the cells in 1 ml 10 mM EDTA pH 8
2	PRE LYSIS Resuspend the pellet in 600 µl sorbitol buffer ³ . Add 50 U lyticase or zymolase ⁴ . Incubate at 30°C for 30 min. This step degrades the yeast cell wall creating spheroplasts (formation of spheroplast 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 BT1. Add 25 µl Proteinase K 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 incubator. Samples can be incubated overnight as well. <i>If RNA-free DNA is crucial for downstream applications, a RNase digest may be performed: Add 20 µl RNase A (20 mg/ml) solution (not included) and incubate for an additional 5 min at room temperature.</i>	 	PELLET 600 µl sorbitol buffer + 50 U lyticase/zymolase Incubate 30°C, 30 min 10 min, 2,000 x g + 180 µl BUFFER BT1 + 25 µl PROTEINASE K Incubate 56°C, 1-3 h/overnight Vortex
3	Proceed with Step 3 of the <i>Standard Protocol</i> .		

F. Protocol for dried blood spots (Guthrie cards)

Before starting with the preparation, set incubators or water baths to 56°C, 70°C and 94°C, respectively. Equilibrate elution Buffer BBE to 70°C.


STEP	DESCRIPTION		
1	PREPARE SAMPLE 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. The area of the dried blood spots should be between 15 and 30 mm ² .		SAMPLE
2	PRE-LYSIS 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 94°C. Let the sample cool down. Add 25 µl Proteinase K, vortex and incubate at 56°C for 1h. Vortex occasionally during incubation or use a shaking water bath. <i>Be sure that the samples are completely covered with lysis buffer during incubation.</i>		+ 180 µl BUFFER BT1 Incubate 94°C, 10 min Cool + 25 µl PROTEINASE K Incubate 56°C, 1 h Vortex
3	SAMPLE LYSIS Add 200 µl Buffer BB3 vortex vigorously and incubate at 56°C for 10 min.		200 µl BUFFER BB3 Vortex Incubate 56°C, 10 min
4	Proceed with Step 4 of the <i>Standard Protocol</i> .		

³ Buffer sorbitol: 1.2 M sorbitol; 10 mM CaCl₂; 0.1 M Tris/HCl pH 7.5; 35 mM β-Mercaptoetanol.

⁴ 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.




G. Protocol for hair roots

Before starting with the preparation, set incubators or water baths to 56°C and 70°C, respectively. Equilibrate elution Buffer BBE to 70°C.

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


H. Protocol from paraffin-embedded tissue

Before starting with the preparation, set incubators or water baths to 37°C, 56°C and 70°C, respectively. Equilibrate elution Buffer BBE to 70°C.

STEP	DESCRIPTION		
1	<p>PREPARE SAMPLE</p> <p>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.</p> <p>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.</p> <p>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.</p> <p>Incubate the open tube at 37°C until the ethanol has evaporated (15 min).</p>	  	<p>SMALL SECTIONS + 1 ml n-OCTANE</p> <p>Vortex Incubate RT, 30 min</p> <p>3 min, 11,000 × g</p> <p>+ 1 ml ETANOL 3 min, 11,000 × g + 1 ml ETANOL 3 min, 11,000 × g</p> <p>Incubate open tube at 37°C</p>
2	Proceed with Step 2 of the <i>Standard Protocol</i> .		

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

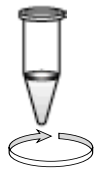
Before starting with the preparation, set incubators or water baths to 56°C and 70°C, respectively. Equilibrate elution Buffer BBE to 70°C. For this protocol depending on the number and the viscosity of the samples you may require and additional bottle of Buffer BT1 (BIOTOOLS Cat. No. 21.161).

STEP	DESCRIPTION		
1	<p>PREPARE SAMPLE</p> <p>Add 200-500 µl sputum or bronchoalveolar lavage to an equal volume N-acetyl cystein/NaOH⁵. Vortex gently to mix.</p> <p>Incubate the mixture for 25 min at room temperature with shaking.</p> <p>Adjust the volume to 25 ml with sterile water.</p> <p>Centrifuge for 30 min at 4,000 x g. Discard the supernatant.</p> <p>Resuspend the pellet in 0.5-1 ml Buffer BT1, depending on sample viscosity (Kit only includes 20 ml of Buffer BT1⁶).</p> <p>Transfer 200 µl of the resuspended sample to a new microcentrifuge tube.</p>		<p>ESPUTUM 200-500 µl + equal vol N-acetyl cystein/NaOH</p> <p>Vortex Incubate RT 25 min</p> <p>Adjust vol to 25 ml with sterile H₂O 30 min, 4,000 x g</p> <p>Resuspend pellet in 0.5-1 ml BUFFER BT1</p> <p>Transfer 200 µl</p>
2	Proceed with Step 2 of the <i>Standard Protocol</i> .		

For detection and quantification of *Mycobacterium tuberculosis* in clinical samples see BIOTOOLS catalogue (BIOTUB-QT Kit in liquid and gel format).

J. Protocol for extraction of genomic DNA from feces

Before starting with the preparation, set incubators or water baths to 56°C and 70°C, respectively. Equilibrate elution Buffer BBE to 70°C. For this protocol depending on the number and the quality of the samples you may require and additional bottle of Buffer BT1 (BIOTOOLS Cat. No. 21.161).

STEP	DESCRIPTION		
1	<p>PREPARE SAMPLE</p> <p>Add 250 mg feces to 1 ml TE buffer⁷: Resuspend the sample by vigorously vortexing (30 s).</p> <p>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.</p> <p><i>The prepared pellet contains, among other constituents, cells from the digestive tract and bacteria.</i></p> <p>Transfer 200 µl of the resuspended sample to a new microcentrifuge tube.</p>		<p>FECES (250 mg) + 1 ml buffer TE</p> <p>Vortex</p> <p>15 min, 4,000 x g</p> <p>Resuspend in 0.2-1 ml BUFFER BT1</p> <p>Transfer 200 µl</p>
2	Proceed with the <i>Standard Protocol</i> with the addition of 25 µl Proteinase K of the Step 2.		

⁵ N-acetyl cystein/NaOH: 2 g NaOH; 1.45 g sodium citrate; 0.5 g N-acetyl cystein. Add water to 100 ml.






⁶ Buffer BT1 Cat. No. 21.161

⁷ Buffer TE: 10 mM Tris/HCl; 1 mM EDTA, pH 8.

K. 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.

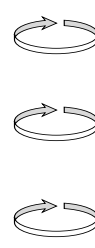
Before starting with the preparation, set incubators or water baths to 56°C and 70°C, respectively. Equilibrate elution Buffer BBE to 70°C.

STEP	DESCRIPTION		
1	<p>PREPARE SAMPLE</p> <p>To a sterile 1 liter flask, add 25 ml milk and 225 ml prewarmed (37°C) mTSB⁸ (supplied with Novobiocin). Incubate the mixture in a shaking water bath for 5-6 h or overnight at 37°C.</p> <p>Centrifuge 100 ml culture for 40 min at 6,000 x g.</p> <p>Gently pour off the supernatant and resuspend the pellet in 2 ml sterile water. Centrifuge for 10 min at 10,000 x g.</p>	 	<p>MILK (25 ml) + 225 ml sterile mTSB</p> <p>Incubate 37°C with shaking, 5-6 h/overnight</p> <p>100 ml culture 40 min, 6,000 x g</p> <p>Resuspend cells in 2 ml sterile H₂O</p> <p>10 min, 10,000 x g</p>
2	<p>PRE-LYSIS</p> <p>Resuspend the pellet in 180 µl Buffer BT1 and add 25 µl Proteinase K.</p>		<p>+ 180 µl BUFFER BT1 + 25 µl PROTEINASE K</p>
3-8	<p>Proceed with Step 3-8 of the <i>Standard Protocol</i>. Perform two elution steps with the indicated elution volume of the <i>Standard Protocol</i>.</p>		<p>200 µl ELUATE</p>
9	<p>After elution of the DNA, proceed with the following step.</p> <p>Precipitate the obtained DNA by adding 20 µl 3.2 M sodium acetate and 400 µl absolute 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.</p> <p><i>The DNA can be used as template for subsequent amplifications</i></p>	 	<p>+ 20 µl 3.2 M SODIUM ACETATE + 400 µl ETHANOL 30 min, 11,000 x g</p> <p>Wash pellet with 1 ml ETHANOL 70%</p> <p>Resuspend in 10 µl sterile H₂O</p>

⁸ mTSB Medium: 30 g Tryptic soy broth (Gibco); 1.5 g biles salts No. 3 (Oxoid); 1.5 g KH₂PO₄. Add 900 ml de 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.




L. Protocol for purification of bacterial DNA e.g. *Borrelia burgdorferi* from urine


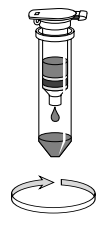

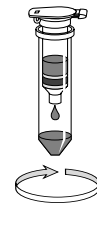
Before starting with the preparation, set incubators or water baths to 56°C and 70°C, respectively. Equilibrate elution Buffer BBE to 70°C.

STEP	DESCRIPTION		
1	<p>PREPARE SAMPLE</p> <p>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.</p> <p><i>The sample material should be fresh and storage at -20 to -80°C is only recommendable for a couple of days. After thawing incubate the sample at 40°C 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</i></p>		<p>1 ml URINE 30 min, 13,000×g</p> <p>+</p> <p>1 ml URINE 30 min, 13,000×g</p> <p>+</p> <p>1 ml URINE 30 min, 13,000×g</p>
2	Proceed with the Step 2 of the <i>Standard Protocol</i> .		

M. Protocol for purification of viral DNA e.g. cytomegalovirus from urine

Before starting with the preparation, set incubator or water bath to 70°C. Equilibrate elution Buffer BBE to 70°C.

STEP	DESCRIPTION		
1	<p>PREPARE SAMPLE</p> <p>Prepare 3-4 aliquots (1 ml each aliquot) of urine sample. Centrifuge aliquots for 10 min at full speed Carefully decant the supernatant of the aliquots.</p> <p><i>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 37-40°C. If a complete solution does not happen let the precipitate sediment and proceed with Step 1 of the protocol using only the supernatant.</i></p>		<p>SAMPLE divided in aliquots of 1 ml</p> <p>1 ml URINE 10 min, max g</p> <p>1 ml URINE 10 min, max g</p> <p>1 ml URINE 10 min, max g</p>
2	<p>PRE-LYSIS</p> <p>There are two possible working procedures:</p> <p>A) Resuspend the first pellet in 180 µl Buffer BT1 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.</p> <p>B) Resuspend each pellet as mentioned above and proceed with Step 3. In this case the solutions are pooled and the spin column has to be loaded successively.</p>		<p>+</p> <p>180 µl BUFFER BT1</p> <p>+</p> <p>25 µl PROTEINASE K</p>
3	<p>SAMPLE LYSIS</p> <p>Add 200 µl lysis Buffer BB3 and incubate at least 20 min at 70°C at least for 20 min. Vortex briefly.</p>		<p>200 µl BUFFER BB3</p> <p>Vortex and Incubate 70°C, 20 min</p>
4	<p>ADJUST DNA BINDING CONDITIONS</p> <p>Add 210 µl ethanol (96-100%) to the sample and vortex vigorously.</p>		<p>+</p> <p>210 µl ETHANOL</p> <p>Vortex</p>

5	BIND DNA For each sample, place one column into a 2 ml centrifuge tube and apply the sample to the column. Centrifuge at $4,500 \times g$ during 1 min. Discard collecting tube with flow-through and place the column back into a new collecting tube.		Load lysate into a column $1 \text{ min, } 4,500 \times g$
6	WASH SILICA MEMBRANE 1st Wash Add 500 μl Buffer BBW. Centrifuge 1 min at $4,500 \times g$. Discard flow-through and place the column back into a new collecting tube. 2nd Wash Add 600 μl Buffer BB5. Centrifuge 2 min at $11,000 \times g$. Discard collecting tube with flow-through.		+ 500 μl BUFFER BBW $1 \text{ min, } 4,500 \times g$ + 600 μl BUFFER BBW $2 \text{ min, } 11,000 \times g$
7	DRY SILICA MEMBRANE Incubate with open lid for 1-2 min at 70°C . Residual ethanol is removed during this step.		Incubate Tubes with open lid $1 \text{ min, } 11,000 \times g$
8	ELUTE HIGHLY PURE DNA Add 70 μl prewarmed elution Buffer BBE (70°C) directly onto the silica membrane close the lid and incubate for further 3-5 min at 70°C . Centrifuge 1 min at $4,500 \times g$.		+ 70 μl BUFFER BBE (70°C) Incubate 70°C 3-5 min $1 \text{ min, } 4,500 \times g$


N. Protocol for purification of genomic DNA from insects

Before starting with the preparation, set incubators or water baths to 56°C and 70°C , respectively. Equilibrate elution Buffer BBE to 70°C .

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



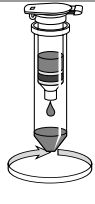
O. Protocol for purification of genomic DNA from dental swabs

Before starting with the preparation, set incubator or water bath to 70°C. Equilibrate elution Buffer BBE to 70°C.

STEP	DESCRIPTION		
1	PREPARE SAMPLE Place swab material (paper, cotton, brushes, plastic) in a 1.5 ml microcentrifuge tube.		SAMPLE
2	PRE-LYSIS 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 get the swab material completely submerged. 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. <i>Depending on the bacterial strains that are to be detected, incubation at 95°C can be skipped.</i>		180 µl BUFFER BT1 + 25 µl PROTEINASE K Spin Incubate 5 min at TA vortex and spin Incubate 10 min, 70°C Incubate 5 min, 95°C Spin
3	Proceed with Step 3 of the <i>Standard Protocol</i> .		

P. Protocol for purification of genomic DNA from buccal swabs

Before starting with the preparation, set incubator or water bath to 70°C. Equilibrate elution Buffer BBE to 70°C.

STEP	DESCRIPTION		
1	PREPARE SAMPLE Collect the samples with cotton, dacron, or C.E.P. swabs. Scrape firmly against the inside of each cheek several times and let the swabs air dry.		SAMPLE
2	PRE-LYSIS Place the dry swab material in 2 ml microcentrifuge tube. Add 400-600 µl PBS ⁹ and 25 µl Proteinase K to the swabs. The volume of PBS is dependent on the type of swab used: for cotton and dacron swabs 400 µl are sufficient; for C.E.P. swabs 600 µl are necessary.		+ 400-600 µl buffer PBS + 25 µl PROTEINASE K
3	SAMPLE LYSIS Add one volume Buffer BB3 (400-600 µl, depending on the swab type) and vortex vigorously. Incubate the samples at 70°C for 10 min.		1 vol BUFFER BB3 Incubate 10 min, 70°C
4	ADJUST DNA BINDING CONDITIONS Add 0.5 volumes (200-300 µl, depending on the swab type) ethanol (96-100%) to each sample and mix by vortexing.		+ 0.5 vol ETHANOL Vortex
5	BIND DNA Transfer 600 µl of the samples from the 2 ml microcentrifuge tubes into the columns. Centrifuge at 11,000 x g for 1 min. If the samples are not drawn through completely, repeat the centrifugation. Discard collecting tube with flow-through and place the column back into a new collecting tube. Repeat Step 5 once or twice, depending on the lysis volume.		Load lysate into a column 1 min, 11,000 x g
6	Proceed with Step 6 of the <i>Standard Protocol</i> .		

⁹ Buffer PBS sterile: dissolve 8 g NaCl; 0.2 g KCl, 1.44 g Na₂HPO₄; 0.24 g KH₂PO₄ in 800 ml H₂O. Adjust pH to 7.4 with HCl. Add H₂O to 1 Liter. Sterilise the Buffer PBS in the autoclave.

8. TROUBLESHOOTING

Problem	Possible cause and suggestions
No yield or poor DNA yield	<p>Incomplete cell lysis</p> <ul style="list-style-type: none"> • Sample not thoroughly homogenized and mixed with Buffer BT1 / Proteinase K. The mixture has to be vortexed vigorously immediately after addition of Buffer BT1. • Decreased Proteinase K activity: store dissolved Proteinase K at 4°C for up to 3 months. Divide the solution into aliquots and store at -20°C if the enzyme will not be used up during this period. <p>Reagents not applied properly</p> <ul style="list-style-type: none"> • Prepare Buffers BB3, BB5, and Proteinase K according to instructions (section 4). Add ethanol to lysates before loading them on the columns. <p>Suboptimal elution of DNA from the column</p> <ul style="list-style-type: none"> • Preheat Buffer BBE to 70°C before elution. Apply Buffer BBE directly onto the center of the silica membrane. • 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). • 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 a incubator at 70°C for 5 min before centrifugation.
Poor DNA quality	<p>Incomplete lysis</p> <ul style="list-style-type: none"> • Sample not thoroughly homogenized and mixed with Buffer BT1 / Proteinase K. The mixture has to be vortexed vigorously immediately after addition of Buffer BT1. • Decreased Proteinase K activity: store dissolved Proteinase K at 4°C for up to 3 months. Divide the solution into aliquots and store at -20°C if the enzyme will not be used up during this period. <p>Reagents not applied properly</p> <ul style="list-style-type: none"> • Prepare Buffers BB3, BB5, and Proteinase K according to instructions (section 4). Add ethanol to lysates before loading them on the columns. <p>RNA in sample</p> <ul style="list-style-type: none"> • If DNA free of RNA is desired, add 10 µl of an RNase A solution (5 mg/ml) before addition of lysis Buffer BB3 and incubate at 37°C for 5 min.

Problem	Possible cause and suggestions
Columns clogged	<p>Too much sample material used</p> <ul style="list-style-type: none"> 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 BT1 and ethanol. <p>Incomplete lysis</p> <ul style="list-style-type: none"> Sample not thoroughly homogenized and mixed with Buffer BT1 / Proteinase K. The mixture has to be vortexed vigorously immediately after addition of Buffer BT1. Decreased Proteinase K activity: store dissolved Proteinase K at 4°C for up to 3 months. Divide the solution into aliquots and store at -20°C if the enzyme will not be used up during this period. <p>Reagents not applied properly</p> <ul style="list-style-type: none"> Prepare Buffers BB3, BB5, and Proteinase K according to instructions (section 4). Add ethanol to lysates before loading them on the columns.
Suboptimal performance of genomic DNA in enzymatic reactions	<p>Carryover of ethanol or salt</p> <ul style="list-style-type: none"> 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. Do not chill Buffer BB5 before use. Cold buffer will not remove salt effectively. Equilibrate Buffer BB5 to room temperature before use. <p>Contamination of DNA with inhibitory substances</p> <ul style="list-style-type: none"> Do not eluate DNA with Tris/EDTA buffer (TE). EDTA may inhibit enzymatic reactions. Repurify DNA and eluate in Buffer BBE. If the $A_{260/280}$ ratio of the eluate is below 1.6, repeat the purification procedure: add 1 volume Buffer BB3 plus 1 volume ethanol (96-100%) to the eluate. Load the mixture onto a column and proceed with step 5 of the <i>Standard Protocol</i>.

9. ORDERING INFORMATION

SPEEDTOOLS KIT	50 PREPS	250 PREPS
SPEEDTOOLS DNA EXTRACTION KIT	Cat. No. 21.131	Cat. No. 21.132
SPEEDTOOLS TISSUE DNA EXTRACTION KIT	Cat. No. 21.136	Cat. No. 21.137
SPEEDTOOLS RNA VIRUS EXTRACTION KIT	Cat. No. 21.141	Cat. No. 21.142
SPEEDTOOLS FOOD DNA EXTRACTION KIT	Cat. No. 21.176	Cat. No. 21.177
SPEEDTOOLS PLANT DNA EXTRACTION KIT	Cat. No. 21.171	Cat. No. 21.172
SPEEDTOOLS TOTAL RNA EXTRACTION KIT	Cat. No. 21.211	Cat. No. 21.212
SPEEDTOOLS PCR CLEAN-UP KIT	Cat. No. 21.201	Cat. No. 21.202
SPEEDTOOLS PLASMID DNA PURIFICATION KIT	Cat. No. 21.221	Cat. No. 21.222

10. PRODUCT USE RESTRICTION AND WARRANTY

- Product for research purposes and *in vitro* uses only.
- No claim or representation is intended for its use to identify any specific microorganism or for clinical use (diagnostic, prognosis, therapeutic, or blood bank).
- 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.
- The kit includes documentation stating specifications and other technical information. Follow the instructions in order to obtain highly pure genomic DNA.
- BIOTOOLS warrants to meet the stated specifications of the kit. Any product not fulfilling the specifications included in the product sheet will be replaced.
- 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.
- BIOTOOLS does not warrant against damages or defects arising in shipping and handling (transport insurance for customers excluded). Any complaint on damaged goods during transport must be directed to the handling or transport agent.
- BIOTOOLS has no responsibility for damages, whether direct or indirect, incidental or consequential of improper or abnormal use of this product. Nor has any responsibility for defects in products or components not manufactured by BIOTOOLS, or against damages resulting from such non-BIOTOOLS components or products.
- BIOTOOLS 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, reproductively, durability, fitness for a particular purpose or use, merchantability, condition, or any other matter with respect to BIOTOOLS products.
- The warranty provided herein and the data, specifications and descriptions of this kit appearing in BIOTOOLS published catalogues and product literature are BIOTOOLS sole representations concerning the product and warranty. No other statements or representations, written or oral, by BIOTOOLS employees, agent or representatives, except written statements signed by a duly authorized officer of BIOTOOLS 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. You may also contact your local distributor for general scientific information.
- Applications mentioned in BIOTOOLS literature are provided for informational purposes only. BIOTOOLS does not warrant that all applications have been tested in BIOTOOLS laboratories using BIOTOOLS products. BIOTOOLS does not warrant the correctness of any of those applications. For more information contact our Technical Dept (info@biotools.eu).

Manufactured by:

BIOTOOLS, Biotechnological & Medical Laboratories, S.A. has been evaluated and certified to accomplish ISO 9001:2000 requirements for the following activities: Research and development of biotechnology products and manufacture of biotechnology and in vitro products.
Valle de Tobalina – 52 – Nave 39, 28021 Madrid – Spain



© 2008 BIOTOOLS, Biotechnological & Medical Laboratories, S.A. All rights reserved.