



# BIOTOOLS

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## **SPEEDTOOLS DNA EXTRACTION KIT**

*Designed for the rapid isolation of highly pure  
genomic DNA from cultured cells, whole blood,  
serum, plasma, or other body fluids*

### **Instructions for Use** (Cat. No. 21.131/2)

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

## 1. BASIC PRINCIPLE

**SPEEDTOOLS DNA EXTRACTION KIT** is designed for the rapid isolation of highly pure genomic DNA from:

- cultured cells
- whole blood
- serum
- plasma
- other body fluids

Lysis of sample is achieved by incubation of sample in a solution containing chaotropic ions in the presence of proteinase K. Addition of ethanol to the lysate create the appropriate conditions for binding of DNA to the silica membrane of the column. This binding process is reversible and specific to nucleic acids. 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

<b>SPEEDTOOLS DNA EXTRACTION KIT</b>	
Cat. No. 21.131	50 Preps
Buffer BB1	10 ml
Reagent BB2	2.5 ml
Buffer BB5 (concentrated)	7 ml
Buffer BBW	30 ml
Buffer BBE	13 ml
Proteinase K (lyophilized)	30 mg
Proteinase Buffer	1.8 ml
DNA Binding Columns	50
2 ml Collecting Tubes	100
Label for Buffer BB3	1
Protocol	1

## 3. INTENDED USE

With the **SPEEDTOOLS DNA** method, genomic DNA is prepared from cultured cells, whole blood, serum, plasma, or other body fluids. It is also possible to purify viral DNA from blood samples. As viral DNA copurifies with cellular DNA, we recommend usage of cell-free sample (serum or plasma) to prepare pure viral DNA.

Blood treated either with EDTA, citrate, or heparin can be used. If leukocyte rich materials like buffy coat are used, apply smaller volumes and dilute the samples with sterile PBS (dissolve 8 g NaCl, 0.2 g KCl, 1.44 g Na<sub>2</sub>HPO<sub>4</sub>, and 0.24 g KH<sub>2</sub>PO<sub>4</sub> in 800 ml H<sub>2</sub>O. Adjust pH to 7.4 with HCl. Add H<sub>2</sub>O to 1 Liter).

The kits allow isolation of highly pure genomic DNA with an A<sub>260</sub>/A<sub>280</sub>-ratio between 1.60 and 1.90 and a typical concentration of 40 – 60 ng per µl.

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	Up to 200 $\mu$ l
Average Yield	4-6 $\mu$ g
Elution Volume	100 $\mu$ l
Binding Capacity	60 $\mu$ g
Time / Prep	30 min
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 DNA EXTRACTION KIT** prepare the following reagents:



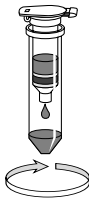


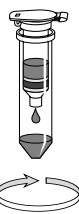
- **Proteinase K:** Add 1.35 ml of Proteinase Buffer to dissolve lyophilized Proteinase K. This solution is stable at +4°C for up to 6 months. Storage at -20°C is recommended if the solution will not be used up during this period.
- **Buffer BB3:** Transfer Buffer BB1 to Reagent BB2 completely and mix 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 Buffer BB3 or Buffer BB1. Dissolve such precipitates by incubation of the bottle at 70°C before use.**

## 5. INSTRUCTION FOR USE

### A. Standard Protocol

Before starting with the preparation, set incubator or water bath to 70°C. Equilibrate Buffer BBE to 70°C. Prepare Buffer BB3, Buffer BB5, and Proteinase K solution (see section 4).

STEP	DESCRIPTION		
1	<p><b>SAMPLE LYSIS</b></p> <ul style="list-style-type: none"> <li>Pipette <b>25 µl Proteinase K</b> and up to <b>200 µl of sample</b> (equilibrated to room temperature) into 1.5 ml microcentrifuge tube.</li> </ul> <p><i>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 <math>5 \times 10^6</math> cells in a final volume of 200 µl PBS.</i></p> <ul style="list-style-type: none"> <li>Add <b>200 µl lysis Buffer BB3</b> to the samples and <b>vortex</b> the mixture vigorously (10-20 s). Incubate samples at <b>70°C</b> for <b>10 - 15 min</b>.</li> </ul> <p><i>The lysate should become brownish during incubation with buffer BB3. Increase incubation time with Proteinase K (up to 30 min) and vortex once or twice vigorously during incubation if processing older or clotted blood samples</i></p>		<p>200 µl SAMPLE + 25 µl PROTEINASE K + 200 µl BUFFER BB3</p> <p>mix 70°C 10-15 min</p>
2	<p><b>ADJUST DNA BINDING CONDITIONS</b></p> <p>Add <b>210 µl ethanol</b> (96-100%) to each sample and <b>vortex</b> again.</p>		<p>+ 210 µl ETHANOL mix</p>
3	<p><b>BIND DNA</b></p> <p>For each preparation, take one <b>column</b> placed in a 2 ml centrifuge tube and <b>load the sample</b>. Centrifuge <b>1 min</b> at <b>11,000 x g</b>. If the samples are not drawn through the matrix completely, repeat the centrifugation at higher g-force (&lt; 15,000 x g). <b>Discard collecting tube with flow-through.</b></p>		<p>Load lysate into a column 1 min, 11,000 x g</p>
4	<p><b>WASH SILICA MEMBRANE</b></p> <p><b>1<sup>st</sup> Wash</b></p> <p>Place the column into a new 2 ml collecting tube and add <b>500 µl Buffer BBW</b>. Centrifuge <b>1 min</b> at <b>11,000 x g</b>. <b>Discard flow-through</b> and reuse the tube.</p> <p><b>2<sup>nd</sup> Wash</b></p> <p>Place the column back into the latter collecting tube and add <b>600 µl Buffer BB5</b>. Centrifuge <b>1 min</b> at <b>11,000 x g</b>. <b>Discard</b> collecting tube with <b>flow-through</b>.</p>		<p>+ 500 µl BUFFER BBW 1 min, 11,000 x g</p> <p>+ 600 µl BUFFER BB5 1 min, 11,000 x g</p>
5	<p><b>DRY SILICA MEMBRANE</b></p> <p>Place the column into 1.5 ml microcentrifuge tube and centrifuge <b>1 min</b> at <b>11,000 x g</b>. Residual ethanol is removed during this step.</p>		<p>1 min, 11,000 x g</p>
6	<p><b>ELUTE HIGHLY PURE DNA</b></p> <p>Place the column in a new 1.5 ml microcentrifuge tube and add <b>100 µl prewarmed elution Buffer BBE (70°C)</b>. Dispense buffer directly onto the silica membrane. Incubate at room temperature for 1 min. Centrifuge <b>1 min</b> at <b>11,000 x g</b>. The eluate contains your pure DNA sample.</p> <p>For alternative elution procedures see section C.</p>		<p>+ 100 µl BUFFER BBE (70°C)</p> <p>Incubate 1 min 1 min, 11,000 x g</p>

## NOTE

For viscous sputum samples is necessary to pre-treat the samples according to the following scheme before using the Speedtools DNA Extraction Kit:

1. Add 1 volume NALC solution to 1 equal volume sample; incubate at room temperature for 20 min.

NALC Solution:

- 1.45 % sodium citrate
- 2 % sodium hydroxide
- 0.5 % N-acetyl-L-cysteine

2. Centrifuge sample at 3500rpm for 20 minutes.


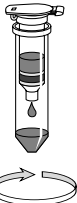
3. Remove and discard supernatant.

4. Resuspend pellet in 1ml 1% PBS.

5. Use 200 µl for Speedtools DNA extraction Kit.

## B. Protocol for extraction and purification of DNA from cytomegalovirus from plasma

Before starting with the preparation, set incubator or water bath to 70°C. Equilibrate Buffer BBE to 70°C. Prepare Buffer BB3, Buffer BB5, and Proteinase K solution (see section 4).

STEP	DESCRIPTION		
1	<b>SAMPLE LYSIS</b> <ul style="list-style-type: none"> <li>• Pipette <b>25 µl Proteinase K</b> and up to <b>250 µl of plasma</b> (equilibrated to room temperature) into 1.5 ml microcentrifuge tube.</li> <li>• Add <b>200 µl lysis Buffer BB3</b> to the samples and <b>vortex</b> the mixture vigorously (10-20 s). Incubate samples at <b>70°C</b> for <b>10 - 15 min</b>.</li> </ul>		250 µl PLASMA + 25 µl PROTEINASE K + 200 µl BUFFER BB3  mix 70°C 10-15 min
2-5	Proceed with Step 2-5 of the <i>Standard Protocol</i> . At Step 3 in order to load the complete lysate perform two loading steps.		
6	<b>ELUTION AND CONCENTRATION OF CYTOMEGALOVIRUS DNA</b> Place the column in a new 1.5 ml microcentrifuge tube and add 40 µl <b>prewarmed elution Buffer BBE (70°C)</b> . Dispense buffer directly onto the silica membrane. Incubate at room temperature for 3 min. Centrifuge <b>1 min at 11,000 x g</b> . The eluate contains your pure DNA sample.		+ 40 µl BUFFER BBE (70°C)  Incubate 3 min  1 min, 11,000 x g

## C. Other Elution Protocols

It is possible to adapt elution method and volume of elution buffer to the subsequent application of interest. In addition to the standard protocol (recovery rate about 70-90%) 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 higher than with standard elution (130%). 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.

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. TROUBLESHOOTING

Problem	Possible cause and suggestions
No yield or poor DNA yield	<p><b>Low concentration of leukocytes in sample</b></p> <ul style="list-style-type: none"> <li>Prepare buffy coat from the blood sample: Centrifuge whole blood at room temperature (3,300 × g; 10 min). Three different layers will be visible after centrifugation. Leukocytes are concentrated in the intermediate layer (buffy coat).</li> </ul> <p><b>Incomplete cell lysis</b></p> <ul style="list-style-type: none"> <li>Sample not thoroughly mixed with lysis buffer / Proteinase K. The mixture has to be vortexed vigorously immediately after addition of lysis buffer.</li> <li>Proteinase K digestion not optimal. Never add Proteinase K directly to lysis buffer. Incubate for 15 - 20 min at 70°C.</li> </ul> <p><b>Reagents not applied properly</b></p> <ul style="list-style-type: none"> <li>Prepare buffers and Proteinase K solution according to instructions (section 4). Add ethanol to lysates before loading them on columns.</li> </ul> <p><b>Suboptimal elution of DNA from the column</b></p> <ul style="list-style-type: none"> <li>Preheat Buffer BBE to 70°C before elution. Apply Buffer BBE directly onto the center of the silica membrane.</li> <li>Elution efficiencies decrease dramatically if elution is performed with buffers of pH &lt; 7.0. Use slightly alkaline elution buffer like Buffer BBE (pH 8.5).</li> <li>Mix vigorously once during the 70°C incubation step especially when working with old or clotted blood samples.</li> </ul>

Problem	Possible cause and suggestions
Suboptimal performance of genomic DNA in enzymatic reactions	<p><b>Carryover of ethanol</b></p> <ul style="list-style-type: none"> <li>Be sure to remove all of ethanolic Buffer BB5 before eluting the DNA. If the level of BB5 after the second wash has reached the column outlet for any reason, discard flow-through, place the column back into the collecting tube, and centrifuge again.</li> </ul> <p><b>Contamination of DNA with inhibitory substances</b></p> <ul style="list-style-type: none"> <li>If DNA has been eluted with Tris/EDTA buffer (TE), make sure that EDTA does not interfere with downstream applications or repurify DNA and elute in BBE buffer.</li> <li>If preparing DNA from older or clotted blood samples, extend Proteinase K incubation to 30 min and vortex once or twice during this step.</li> <li>If the <math>A_{260/280}</math>-ratio of the eluate is below 1.6, repeat the purification procedure: Add 1 volume of Buffer BB3 plus 1 volume ethanol to the eluate, load on column, and proceed with step 3 of the corresponding protocol.</li> </ul>
Poor DNA quality	<p><b>Reagents not applied properly</b></p> <ul style="list-style-type: none"> <li>Prepare buffers and Proteinase K solution according to instructions (section 4). Add ethanol to lysates and mix before loading them on columns.</li> </ul> <p><b>Incomplete cell lysis</b></p> <ul style="list-style-type: none"> <li>Sample not thoroughly mixed with lysis buffer / Proteinase K. The mixture has to be vortexed vigorously immediately after addition of lysis buffer.</li> <li>Proteinase K digestion not optimal. Never add Proteinase K directly to lysis buffer. Incubate for 15 - 20 min at 70°C.</li> </ul> <p><b>RNA in sample</b></p> <ul style="list-style-type: none"> <li>If DNA free of RNA is desired, add 20 <math>\mu</math>l of an RNase A solution (20 mg/ml) before addition of lysis buffer.</li> </ul> <p><b>Old or clotted blood samples processed</b></p> <ul style="list-style-type: none"> <li>For isolation of DNA from older or clotted blood samples, we recommend extension of Proteinase K incubation to 30 min and vortexing several times during this step.</li> </ul>
Suboptimal performance of genomic DNA in enzymatic reactions	<p><b>Carryover of ethanol</b></p> <ul style="list-style-type: none"> <li>Be sure to remove all of ethanolic Buffer BB5 before eluting the DNA. If the level of BB5 after the second wash has reached the column outlet for any reason, discard flow-through, place the column back into the collecting tube, and centrifuge again.</li> </ul> <p><b>Contamination of DNA with inhibitory substances</b></p> <ul style="list-style-type: none"> <li>If DNA has been eluted with Tris/EDTA buffer (TE), make sure that EDTA does not interfere with downstream applications or repurify DNA and elute in BBE buffer.</li> <li>If preparing DNA from older or clotted blood samples, extend Proteinase K incubation to 30 min and vortex once or twice during this step.</li> <li>If the <math>A_{260/280}</math>-ratio of the eluate is below 1.6, repeat the purification procedure: Add 1 volume of Buffer BB3 plus 1 volume ethanol to the eluate, load on column, and proceed with step 3 of the corresponding protocol.</li> </ul>

## 7. SAFETY INSTRUCTIONS

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

Reagent	Hazard Contents	Hazard Symbol	Risk Phrases	Safety Phrases
BB1	Guanidine hydrochloride	<b>X<sup>m</sup></b> <b>Xn<sup>*</sup></b>	<u>Harmful if swallowed.</u> <u>Irritating to eyes and skin</u> <u>Do not breathe dust</u>	<u>R 22-36/38</u> <u>S 22</u>
BBW	Guanidine hydrochloride	<b>X<sup>m</sup></b> <b>Xn<sup>*</sup></b>	<u>Harmful if swallowed</u> <u>Irritating to eyes and skin</u> <u>Do not breathe dust</u>	<u>R 22-36/38</u> <u>S 22</u>
Proteinase K	Proteinase K, lyophilised	<b>X<sup>m</sup></b> <b>Xn<sup>*</sup></b>	<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>	<u>R 36/37/38-42</u> <u>S 22-24-26-36/37</u>

## 8. ORDERING INFORMATION

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

\* 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)

## 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.
7. 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.
8. 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.
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### 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.  
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