



# BIOTOOLS

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## **SPEEDTOOLS RNA VIRUS EXTRACTION KIT**

*Designed for the rapid isolation of highly pure  
viral nucleic acids from cell-free biological fluids  
(not for blood samples)*

### **Instructions for Use** (Cat. No. 21.141/2)

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

## 1. BASIC PRINCIPLE

**SPEEDTOOLS RNA VIRUS EXTRACTION KIT** is designed for the rapid preparation of highly pure viral nucleic acids (e.g. HCV, HIV, CMV) from cell-free biological fluids (but not blood):

- plasma
- serum
- urine
- other body fluids

In a first step the sample containing RNA viruses is lysed by incubation in a lysis solution (Buffer BAV1) containing guanidine isothiocyanate (RNase inhibitor). Lysis buffer and ethanol create the appropriate conditions for binding of nucleic acids to the silica membrane of the column. This binding process is reversible and specific to nucleic acids. Carrier RNA improves binding and recovery of the low-concentrated viral RNA. Contaminations (potential PCR inhibitors) like salts, metabolites and soluble macromolecular cellular components are removed in simple washing steps with ethanolic buffers BAW and BAV3. In a final elution step the nucleic acids can be eluted in low salt buffer or water and are ready for use in subsequent reactions.

DNA virus (e.g. HBV) are usually more difficult to isolate and require proteinase K digestion step. This user manual contains a support protocol for isolation of viral DNA (proteinase K solution is not included).

## 2. KIT CONTENTS

Buffers and SPEEDTOOLS RNA Virus binding columns have been tested with rRNA and MS2 phage RNA. The absence of RNases, and the yield and efficiency of purification have been investigated with RT-PCR.

<b>SPEEDTOOLS RNA VIRUS EXTRACTION KIT</b>	
<b>Cat. No. 21.141</b>	<b>50 Preps</b>
Buffer BAV1	35 ml
Buffer BAW	30 ml
Buffer BAV3 (concentrated)	12.5 ml
RNase-free H <sub>2</sub> O	5 ml
Buffer BRE	5 ml
Carrier RNA (lyophilized)	1 mg
RNA Virus Binding Columns	50
2 ml Collecting Tubes	200
Protocol	1

## 3. INTENDED USE

With the **SPEEDTOOLS RNA VIRUS EXTRACTION** method, viral nucleic acids are isolated and purified from fluid biological samples e.g. plasma, serum, urine but not blood. Due to closed systems no cross contamination occurs. The prepared nucleic acids are suitable for applications like automated fluorescent nucleic acids sequencing, RT-PCR, or any kind of enzymatic manipulation.

The detection limit for certain viruses depends on individual detection procedures e.g. in-house nested (RT-) PCR. We highly recommend the use of internal (low-copy) standards as well as positive and negative controls in order to monitor the purification, amplification and detection processes.

All kinds of biological fluids or semi-fluid samples can be processed. For successful nucleic acid purification, **it is important to obtain a homogeneous, clear and non-viscous sample before loading onto the RNA virus binding columns.** Therefore, check samples (especially old or frozen ones) for the presence of precipitates. **Avoid clearing samples by centrifugation/filtration** before the lysis step with Buffer BAV1, because viruses of interest may be associated with particles or aggregates. Incubation with lysed buffer can be prolonged in order to dissolve and digest residual cell structures, precipitates and virus particles. However, RNA is rather sensitive, and prolonged incubation may cause decreased yields.

**Table 1: General Characteristics of the Kit**

RNA Virus	
Sample Size	Up to 150 µl
Typical Recovery Rates	> 90%
Typical Analysis Limit	30-60 copies/ ml*
Elution Volume	50 µl
Binding Capacity	40 µg
Time / Prep	30 min / 4-6
Spin Column Type	mini

#### 4. PREPARATION OF WORKING SOLUTIONS AND STORAGE CONDITIONS

##### NOTE

*Buffers BAV1 and BAW contain guanidine salts therefore wear gloves and goggles.*

All kit components can be stored at room temperature (20-25°C) and are stable up to one year. Carrier RNA has a limited shelf life in Buffer BAV1. For this reason, some kits contain several bottles of lyophilized carrier RNA that should be used successively as required, to avoid degradation of carrier RNA.

Before starting any protocol with **SPEEDTOOLS RNA VIRUS EXTRACTION KIT** prepare the following reagents:

- **Buffer BAV1(including carrier RNA):** add 1 ml of lysis buffer BAV1 to the carrier RNA lyophilized tube. Dissolve the complete contents of the carrier RNA tube and transfer it back to the Buffer BAV1 bottle. Buffer BAV1 including carrier RNA can be stored **at room temperature for 1-2 weeks.** Storage at room temperature prevents salt precipitation and avoids prewarming the buffer. It can also be stored **at 4°C for up to 4 weeks** or **aliquoted and stored at -20°C for longer periods.** Storage at 4°C or below may cause salt precipitation. Therefore, the mixture must be prewarmed at 40-60°C for a maximum of 5 min in order to redissolve salts.

##### NOTE

*Do not warm Buffer BAV1 containing carrier RNA more than 4 times. Frequent warming, temperatures >80°C and extended heat incubation will accelerate the degradation of carrier RNA. This leads to reduced recovery of viral RNA and eventually false negative RT-PCR results, in particular if low-titer samples are used.*



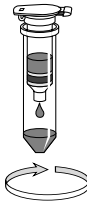
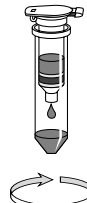
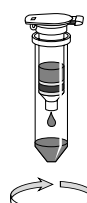
- **Buffer BAV3:** Add 50 ml ethanol (96-100%) to concentrated buffer BAV3 as indicated on the bottle. Store Buffer BAV3 at room temperature for up to one year.

\* nested PCR

## 5. INSTRUCTION FOR USE

### A. Protocol for Isolation of Viral RNA from Cell-Free Biological Fluids


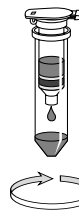
Before starting the protocol, prepare a 70°C incubation block and preheat an aliquot of elution buffer/water.

STEP	DESCRIPTION		
1	<p><b>LYSIS OF VIRUS</b></p> <p>Add <b>600 µl BUFFER BAV1</b> containing carrier RNA to <b>150 µl of the fluid sample</b> into 2 ml microcentrifuge tube. Pipette mixture up and down and <b>vortex well</b>. Incubate for <b>5 min at 70°C</b>.</p> <p><i>Incubation time and temperature are critical for lysis as well as RNA stability (see troubleshooting for further hints).</i></p> <p><i>If the resulting solution is still turbid, centrifuge the mixture for 1 min at 11,000 x g to pellet particles (to prevent clogging of the binding column). Take off the supernatant and proceed with the step 2.</i></p>		<p><b>150 µl SAMPLE</b> + <b>600 µl BUFFER BAV1</b></p> <p><b>vortex</b> 70°C 5 min</p>
2	<p><b>ADJUST BINDING CONDITIONS</b></p> <p>Add <b>600 µl ethanol (96-100%)</b> to the clear lysis solution and mix by <b>vortexing</b> (10-15 sec).</p>		<p>+ <b>600 µl ETHANOL</b> <b>vortex</b></p>
3	<p><b>BIND VIRAL RNA</b></p> <p>Place <b>column</b> in a 2 ml centrifuge tube and <b>load 700 µl lysed sample</b>. Centrifuge <b>1 min at 8,000 x g</b>.</p> <p><i>The use of new 2 ml collecting tubes for every step is recommended if infectious material has to be prepared. This avoids cross-contamination and contamination of centrifuge units. For non-infectious samples, we recommended to discard the flow-through and reuse the 2 ml tube for loading and washing steps.</i></p> <p><b>Load the residual lysed sample</b> onto the column. Centrifuge <b>1 min at 8,000 x g</b>. <b>Discard the flow-through</b> and put the column into another 2 ml collecting tube. More than two loading steps are nor recommended.</p>		<p><b>Load lysate</b> <b>stepwise into a column</b></p> <p>1 min, 8,000 x g</p>
4	<p><b>WASH AND DRY SILICA MEMBRANE</b></p> <p><b>1<sup>st</sup> Wash</b></p> <p>Add 500 µl Buffer BAW to the column. Centrifuge for 1 min at 8,000 x g. Discard flow-through and place the column into a new 2 ml collecting tube.</p> <p>Washing step removes contaminants and PCR inhibitors.</p> <p><b>2<sup>nd</sup> Wash</b></p> <p>Add 600 µl Buffer BAV3 to the column. Centrifuge for 1 min at 8,000 x g. Discard flow-through and place the column into a new 2 ml collecting tube.</p> <p><b>3<sup>rd</sup> Wash</b></p> <p><b>Add 200 µl Buffer BAV3. Centrifuge for 2-5 min at 11,000 x g to remove ethanolic Buffer BAV3 completely.</b> Discard flow-through.</p> <p>Optional: Residual Buffer BAV3 may inhibit subsequent reactions. Therefore, for subsequent reactions, which are extremely ethanol-sensitive, we recommend to repeat the centrifugation with a new 2ml collection tube. Or alternatively, to incubate the column for 1 min at 70°C to remove any remaining traces of ethanol.</p>		<p>+ <b>500 µl BUFFER BAW</b> 1 min, 8,000 x g</p> <p>+ <b>600 µl BUFFER BAV3</b> 1 min, 8,000 x g</p> <p>+ <b>200 µl BUFFER BAV3</b> 5 min, 11,000 x g</p>
5	<p><b>ELUTE VIRAL RNA</b></p> <p><b>Place the column in a new 1.5 ml microcentrifuge tube and add 50 µl RNase-free water</b> (preheated to 70°C). <b>Dispense buffer directly onto the silica membrane</b>. Incubate at room temperature for 1-2 min. <b>Centrifuge 1 min at 11,000 x g</b>. The eluate contains your pure viral RNA.</p> <p>For alternative elution procedures see section C.</p>		<p>+ <b>50 µl RNase-free H<sub>2</sub>O</b> <b>(70°C)</b></p> <p><b>Incubate</b> <b>2 min</b></p> <p>1 min, 11,000 x g</p>

## B. Support Protocol for Isolation of Viral RNA and DNA from Cell-free Biological Fluids

This standard protocol is recommended for the purification of viral RNA and all types of DNA viruses like HBV and CMV (for detection of CMV see BIOCMV QT Kit, Biotoools Cat. No. 90.501/2 liquid form, 90.505/6 gel form).

Before starting the viral nucleic acid isolation, prepare a 70°C incubation block and preheat an aliquot of elution buffer/water. We recommend to use Buffer BRE preheated at 70°C as elution buffer at step 5 of the protocol (also see section C).

STEP	DESCRIPTION		
1	<p><b>LYSIS OF VIRUS</b></p> <p>Add <b>600 µl Buffer BAV1</b> containing carrier RNA to <b>150 µl of the fluid sample</b> into 2 ml microcentrifuge tube. Add <b>20 µl Proteinase K</b> (20 mg/ml stock solution), to the lysis mixture. Pipette mixture up and down and vortex well. Incubate for <b>5 min at 70°C</b>.</p> <p><i>Incubation time and temperature are critical for lysis as well as RNA stability (see troubleshooting for further hints).</i></p> <p><i>If the resulting solution is still turbid, centrifuge the mixture for 1 min at 11,000 x g to pellet particles (to prevent clogging of the binding column). Take off the supernatant and continue with the step 2 to 4 of the protocol A.</i></p>		<p><b>150 µl SAMPLE</b> + <b>600 µl BUFFER BAV1</b> + <b>20 µl PROTEINASE K</b></p> <p><b>vortex</b> 70°C 5 min</p>
2 - 4	Repeat steps 2, 3 and 4 as described in Protocol A.		
5	<p><b>ELUTE VIRAL NUCLEIC ACIDS</b></p> <p>Place the column in a new 1.5 ml microcentrifuge tube and add 50 µl Buffer BRE (preheated to 70°C). Dispense buffer directly onto the silica membrane. Incubate at room temperature for 1-2 min. Centrifuge 1 min at 11,000 x g. The eluate contains your pure viral nucleic acids.</p> <p>For alternative elution procedures see section C.</p>		<p>+ <b>50 µl BUFFER BRE (70°C)</b></p> <p><b>Incubate 2 min</b></p> <p><b>1 min 11,000 x g</b></p>

## C. Other Elution Protocols

- Pure nucleic acids are finally eluted under low ionic strength conditions with nuclease-free water (pH about 7-8) or Buffer BRE which is slightly alkaline (5 mM Tris-HCl, pH 8.5).
- Elution can be done once with water/elution buffer as indicated in the protocol obtaining at least 80% of the bound nucleic acids. To improve sensitivity, this eluate can be used in a second elution step, increasing the efficiency of elution and the concentration of viral nucleic acids slightly. Alternatively, a second elution can be done with an additional volume of water/elution buffer releasing nearly all the bound nucleic acids but resulting in a lower concentrated combined eluate.
- RNA should be eluted with the water supplied, and DNA with elution Buffer BRE which provides better storage conditions for DNA. To elute both types of nucleic acids together use the pH proofed (pH 6-8), nuclease-free water preheated to 70°C.

## 6. TROUBLESHOOTING

Problem	Possible cause and suggestions
Small amounts or no viral nucleic acids in the eluate	<p><b>Problem with carrier RNA</b></p> <ul style="list-style-type: none"> <li>Carrier RNA not added.</li> <li>See remarks concerning storage of Buffer BAV1 with carrier RNA (section 4).</li> </ul> <p><b>Proteinase K digestion may be necessary</b></p> <ul style="list-style-type: none"> <li>Use and compare protocols with and without Proteinase K digestion or prolong incubation time to 10 min.</li> </ul> <p><b>Viral nucleic acids degraded</b></p> <ul style="list-style-type: none"> <li>Samples should be processed immediately. If necessary, add RNase inhibitor to the sample and ensure appropriate storage conditions up to the processing.</li> <li>Check that all buffers have been prepared and stored correctly. If in doubt, use new aliquots of Buffer BAV1, carrier RNA and elution buffer.</li> </ul>
Problems with subsequent detection	<p><b>Reduced sensitivity</b></p> <ul style="list-style-type: none"> <li>Change the volume of eluate added to the PCR/RT-PCR.</li> <li>Incubation time and temperature are critical for lysis as well as RNA stability. For sensitive RNA preparations, incubation at room temperature is sufficient without significant loss of sensitivity. For parallel isolation of viral RNA and DNA, incubation time (5-15 min) and temperature (RT/ 56°C/ 72°C) may be adapted in order to get optimal recovery rates for both species.</li> </ul> <p><b>Ethanol carryover</b></p> <ul style="list-style-type: none"> <li>Prolong centrifugation steps in order to remove Buffer BAV3 completely.</li> </ul>
General problems	<p><b>Clogged membrane</b></p> <ul style="list-style-type: none"> <li>Centrifuge plasma lysate before the addition of ethanol and subsequent loading onto the corresponding columns.</li> </ul>

## 7. SAFETY INSTRUCTIONS

The following components of the SPEEDTOOLS RNA VIRUS 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
BAV1	<u>Guanidine thiocyanate</u>	<b>X<sup>n</sup></b> <u>Xn<sup>*</sup></u>	<u>Harmful by inhalation, in contact with skin and if swallowed</u> <u>Keep away from food, drink and animal</u>	<u>R 20/21/22</u> <u>S 13</u>
BAW	<u>Guanidine hydrochloride</u>	<b>X<sup>n</sup></b> <u>Xn</u>	<u>Flammable.</u> <u>Harmful if swallowed.</u> <u>Irritating to eyes, respiratory system and skin</u> <u>Keep container tightly closed</u> <u>Keep away from sources of ignition - No Smoking!</u> <u>Do not breathe dust</u>	<u>R 10-22-36/38</u> <u>S 7-16-22</u>

## 8. ORDERING INFORMATION

SPEEDTOOLS RNA VIRUS EXTRACTION KIT	Cat. No. 21.141/2	50/250 preps
SPEEDTOOLS DNA EXTRACTION KIT	Cat. No. 21.131/2	50/250 preps
SPEEDTOOLS TISSUE DNA EXTRACTION KIT	Cat. No. 21.136/7	50/250 preps
SPEEDTOOLS FOOD DNA EXTRACTION KIT	Cat. No. 21.176/7	50/250 preps
SPEEDTOOLS PLANT DNA EXTRACTION KIT	Cat. No. 21.171/2	50/250 preps
SPEEDTOOLS PCR CLEAN-UP KIT	Cat. No. 21.201/2	50/250 preps

\* 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 viral nucleic acids.
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.
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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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