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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

Instructions for Use

(Ref.21.140M/1/2)

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 from fluid biological samples:

- plasma
- serum
- urine
- blood
- tissues

In a first step the sample containing RNA viruses is lysed by incubation in a BUFFER BAV1 containing guanidine isothiocyanate (RNase inhibitor). Lysis buffer and ethanol create the appropriate conditions for binding of nucleic acids to the silica membrane including in spin columns. This binding process is reversible and specific to nucleic acids. Carrier RNA improves binding and recovery of the low-concentrated viral RNA. Contaminants like salts, metabolites and soluble macromolecular cellular components are removed in simple washing steps with BUFFER BAW and BUFFER BAV3. In a final elution step the nucleic acids can be eluted in low salt buffer or water.

Viral DNA is difficult to lyse and require proteinase K digestion step.

2. KIT CONTENTS

The absence of RNases, and the yield and efficiency of purification have been investigated with RT-PCR.

Speedtools RNA Virus Extraction kit			
Reagents	**10 Preps Ref. 21.140M**	**50 Preps Ref. 21.141**	**250 Preps Ref. 21.142**
BUFFER BAV1 Lysis Buffer	10 mL	35 mL	5 x 35 mL
BUFFER BAW Wash Buffer	6 mL	30 mL	5 x 30 mL
BUFFER BAV3 (concentrate) Wash Buffer	6 mL	12 mL	5 x 12 mL
RNase-free Water	13 mL	13 mL	5 x 13 mL
BUFFER BRE Elution Buffer	13 mL	13 mL	5 x 13 mL
CARRIER RNA (lyophilized)	300 µg	1 mg	5 x 1 mg
RNA VIRUS COLUMNS	10	50	5 x 50
COLLECTION TUBES	30	150	5 x 150
USER MANUAL	1	1	5 x 1

3. INTENDED USE

With the **SPEEDTOOLS RNA VIRUS EXTRACTION** method, viral nucleic acids are isolated and purified from **150 µL** of fluid biological samples (e.g. plasma, serum, urine, etc). 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.

Carrier RNA enhances binding of viral nucleic acids to the silica membrane and reduces the risk of viral RNA degradation. Final eluates contain both viral nucleic acids and Carrier RNA; therefore, it is not possible to quantify the nucleic acids isolated with the kit by photometric or fluorometric methods when using the carrier. Other methods of quantification such as specific quantitative PCR or RT-PCR systems are recommended.

General Characteristics of the Kit	
Sample material	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 preps
Spin Column	mini

*serum, plasma, cell-free biological fluids
**Nested PCR

4. SAMPLE QUALITY AND PREPARATION

Liquid samples

Biological fluids or semi-fluid samples can be processed with the kit (e.g., serum, urine, bronchoalveolar lavage). For successful nucleic acid purification it is important to obtain a homogeneous, clear, and non-viscous sample before loading onto the RNA Virus Binding Column; check all samples (especially old or frozen ones) for presence of precipitates. Precipitates remaining after lysis with Buffer BAV1 can be removed by centrifugation.

Since viruses may be associated with particles or aggregates, avoid clearing samples before lysis. Incubation with Lysis Buffer BAV1 can be prolonged in order to dissolve and digest residual cell structures, precipitates and virus particles. RNA, however, is sensitive and prolonged incubation may cause decreased yields.

Solid samples (e.g. tissue samples, stool samples)

Prepare a 10 % (w/v) suspension of tissue in a suitable buffer (e.g., PBS) using commercial homogenization tools (rotor-stator or bead-based homogenization tools, etc.). Centrifuge the suspension in order to remove cellular debris. Use the clear particle-free supernatant for further processing.

Swab material

Incubate swab in a suitable buffer (e.g., PBS) or cell-culture medium for 30 min.
Proceed with particle-free buffer or medium.

Blood samples

Processing of blood samples is possible if using blood diluted with PBS buffer. Using undiluted blood may cause clogging of the silica membrane of the RNA Virus Binding Column.

The amount of PBS buffer added to blood samples has to be optimized for the individual organism. As a rule of thumb we recommend to start with 50 µl of blood diluted with 50 µl of PBS buffer.

Proteinase K treatment

Addition of Proteinase K solution (not provided with the kit) is necessary for the isolation of viral DNA or simultaneous viral RNA/DNA isolation.

Although for isolation of viral RNA, Proteinase K treatment is usually not required, it is recommended for viral RNA isolation from viscous samples (e.g., sputum samples).

Sample lysis

For isolation of **viral RNA** in general a lysis of samples in Buffer BAV1 for **10 min at room temperature** (20–25 °C) will be sufficient.

For isolation of **viral RNA from viscous samples**, for example sputum or supernatants of tissue suspensions or stool samples, a lysis at **70 °C** may be required.

For simultaneous **isolation of viral RNA and DNA**, incubation time (**5–15 min**), and temperature (RT, 56 °C, or 70 °C) should be optimized and adjusted to the sample material used.

5. DNA ELUTION PROCEDURES

- Pure nucleic acids are finally eluted under low ionic strength conditions with nuclease-free water (**pH about 7-8**) or Elution 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 **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. PREPARATION OF 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 (18-25°C) through the expiration date printed on the packaging label.
- Carrier RNA has a limited shelf life in Buffer BAV1. *Due to the production procedure and the small amount of Carrier RNA contained in the vial, the carrier may hardly be visible in the vial.*

Before starting any protocol with prepare the following reagents:

I. **<u>ARN carrier</u>:**

- ✓ **10 Preps Format:** Add **1 mL of Buffer BAV1 to the carrier RNA** (lyophilized). Dissolve the complete contents of the carrier RNA tube and transfer it back to the Buffer BAV1.
- ✓ **50 Preps Format:** Add **1 mL of Buffer BAV1 to the carrier RNA** (lyophilized). Dissolve the complete contents of the carrier RNA tube and transfer it back to the Buffer BAV1.

Buffer BAV1 including carrier RNA can be stored ***at room temperature for 1-2 weeks****. Storage at room temperature prevents salt precipitation and avoids pre-warm 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 4ºC or below may cause salt precipitation. Therefore, the mixture must be pre-warmed at 40-60°C for a maximum of 5 min in order to re-dissolve 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-titter samples are used.***

II. <u>Buffer BAV3:</u>

- ✓ **10 Preps Format:** Add **24 mL of ethanol** (96-100%) to the concentrated Buffer BAV3. Mark the label of the bottle to indicate that ethanol is added.
- ✓ **50 Preps Format:** Add **48 mL of ethanol** (96-100%) to the concentrated Buffer BAV3. Mark the label of the bottle to indicate that ethanol is added.

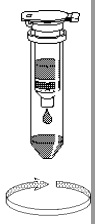
The diluted buffer is stable for up to 12 months at room temperature (18-25°C).

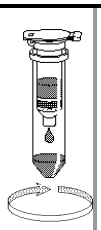
7. INSTRUCTION FOR USE

A. Viral RNA Isolation from Cell-free Biological Fluids

Before starting the preparation:

- Check if Lysis Buffer BAV1 and Wash Buffer BAV3 were prepared according to Section 6.
- Preheat an aliquot of RNase-free water to 70ºC.
- Set an incubator or water bath to 70 ºC.

STEP	DESCRIPTION		
1	**LYSIS OF VIRUS** Add **600 µL BUFFER BAV1** containing carrier RNA to **150 µL of the fluid sample** into a microcentrifuge tube. Pippette mixture up and down and **vortex** well. Incubate for **5 min at 70ºC**. *Incubation time and temperature are critical for lysis as well as RNA stability (Section 4).* *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.*		150 µL SAMPLE + 600 µL BUFFER BAV1 vortex 70°C 5 min
2	**ADJUST BINDING CONDITIONS** Add **600 µL of ethanol (96-100%)** to the clear lysis solution and mix by **vortexing** (10-15 sec).		+ 600 µL ETHANOL vortex
3	**BIND VIRAL RNA** Placed RNA Virus Binding Column in a 2 ml Collection tube and **load 700 µL lysed sample**. Centrifuge **1 min** at **8,000 × *g***. *The use of new 2 ml collection tubes for every step is recommended if infectious material has to be prepared.* **Load the residual lysed sample** onto the column. Centrifuge **1 min at 8,000 × *g***. **Discard the flow-through** and place the column into another 2 ml collection tube. More than two loading steps are not recommended.		Load lysate stepwise into a column *1 min, 8,000 × g*
4	**WASH AND DRY SILICA MEMBRANE** **1st Wash** 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 collection tube. **2nd Wash** 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 collection tube. **3rd Wash** Add **200 µL Buffer BAV3**. Centrifuge for **2-5 min** at **11,000 x g** to remove ethanolic Buffer BAV3 completely. Discard flow-through. *Washing step removes contaminants and PCR inhibitors.* ***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 70ºC to remove any remaining traces of ethanol.*		+ 500 µL BUFFER BAW *1 min, 8,000 × g* + 600 µL BUFFER BAV3 *1 min, 8,000 × g* + 200 µL BUFFER BAV3 *5 min, 11,000 × g*

5	**ELUTE VIRAL RNA** Place the column in a new 1.5 ml microcentrifuge tube and add **50 µL RNase-free water** (preheated to 70°C). Dispense buffer directly onto the silica membrane. Incubate at **room temperatur**e for **1-2 min**. Centrifuge **1 min** at **11,000 x g**. The eluate contains your pure viral RNA. ***For alternative elution procedures see Section 5.***		+ 50 µL RNase-free H_2O (70ºC) Incubate 2 min *1 min, 11,000 × g*

B. Isolation of Viral RNA and DNA from Cell-free Biological Fluids

This standard protocol is recommended for the purification of viral RNA and viral DNA from small samples (< 150 µl).

Before starting the preparation:

- Check if Lysis Buffer BAV1 and Wash Buffer BAV3 were prepared according to Section 6.
- Check if Proteinase K solution (not provided with the kit) was prepared.
- Preheat an aliquot of RNase-free water to 70ºC.
- Set an incubator or water bath to 70 ºC.

STEP	DESCRIPTION		
1	**LYSIS OF VIRUS** Add **600 µL Buffer BAV1** containing carrier RNA to **150 µL of the fluid sample** into 2 ml microcentrifuge tube. Add **20 µL Proteinase K** (20 mg/ml stock solution), to the lysis mixture. Pippette mixture up and down and vortex well. Incubate for **5 min at 70ºC**. *Incubation time and temperature are critical for lysis as well as RNA stability (see troubleshooting for further hints).* *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 supernatant and continue with the protocol.*		150 µL SAMPLE + 600 µL BUFFER BAV1 + 20 µL Proteinase K vortex 70°C 5 min
2	**Proceed with Step 2 of the Protocol A.**		

8. TROUBLESHOOTING

Problem	Possible cause and suggestions
Small amounts or no viral nucleic acids in the eluate	***Problem with carrier RNA*** • Carrier RNA not added. • See remarks concerning storage of Buffer BAV1 with carrier RNA (Section 6). ***Proteinase K digestion may be necessary*** • Use and compare protocols with and without Proteinase K digestion or prolong incubation time to 10 min. ***Viral nucleic acids degraded*** • Samples should be processed immediately. If necessary, add RNase inhibitor to the sample and ensure appropriate storage conditions up to the processing. • Check that all buffers have been prepared and stored correctly. If in doubt, use new aliquots of Buffer BAV1, carrier RNA and Elution Buffer BRE.
Problems with subsequent detection	***Reduced sensitivity*** • Change the volume of eluate added to the PCR/RT-PCR. • 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. ***Ethanol carryover*** • Prolong centrifugation steps in order to remove Buffer BAV3 completely.
General problems	***Clogged membrane*** • Centrifuge plasma lysate before the addition of ethanol and subsequent loading onto the corresponding RNA Virus Binding Column.

9. 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

10. 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.
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