GeneProof
PathogenFree RNA Isolation Kit

User Manual

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1 Components

1.1 Kit contents

	GeneProof PathogenFree RNA Isolation Kit	
Cat. No.	**50 preps**	**250 preps**
Lysis Buffer RAV1	35 ml	5 x 35 ml
Wash Buffer RAW	30 ml	2 x 75 ml
Wash Buffer RAV3 (Concentrate)*	12.5 ml	3 x 25 ml
RNase-free H2O	5 ml	25 ml
Elution Buffer RE**	5 ml	25 ml
Carrier RNA (lyophilized)	1 mg	5 x 1 mg
RNA Virus Columns (dark blue rings, plus Collection Tubes)	50	250
Collection Tubes (2 ml)	200	1000
User Manual	1	1

* For preparation of working solutions and storage conditions see section 3.
** Composition of Elution Buffer RE: 5 mM Tris/HCl, pH 8.5

1.2 Reagents, consumables, and equipment to be supplied by user

Reagents

- 96-100% ethanol

Consumables

- 1.5 ml micro centrifuge tubes
- Disposable tips

Equipment

- Manual pipettes
- Centrifuge for micro centrifuge tubes
- Vortex mixer
- Heating block or water bath for 70°C incubation
- Personal protection equipment (lab coat, gloves, goggles)

2 Product description

2.1 The basic principle

With the **GeneProof PathogenFree RNA Isolation Kit** method, RNA viruses are lysed quickly and efficiently by Lysis Buffer RAV1 which is a highly concentrated solution of GITC. DNA viruses (e.g. HBV) are usually more difficult to lyse and require Proteinase K digestion (see support protocol, section 5.2). Lysis buffer and ethanol create appropriate conditions for binding of nucleic acids to the silica membrane of the **RNA Virus Columns**. Carrier RNA improves binding and recovery of 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 **RAW** and **RAV3**. The nucleic acids can be eluted in low salt buffer or water and are ready-for-use in subsequent reactions.

2.2 Kit specifications

- **GeneProof PathogenFree RNA Isolation kits** are designed for the rapid preparation of highly pure viral nucleic acids (e.g. HCV, HIV, CMV) from fluid biological samples e.g. plasma, serum, urine, but not blood.
- No cross contamination due to closed systems.
- The prepared nucleic acids are suitable for applications like automated fluorescent DNA sequencing, RT-PCR, or any kind of enzymatic reaction. The detection limit for certain viruses depends on the individual procedures e.g. in-house nested (RT-)PCR. We highly recommend using internal (low-copy) standards as well as positive and negative controls to monitor the purification, amplification, and detection processes.

Table 1: Kit specification	GeneProof RNA Isolation Kit
Parameter	
Sample size	Up to 150 µl
Typical recovery rates	> 90%
Typical analysis limit	30 -60 cp/ml
Elution volume	50 µl
Binding capacity	40 µg
Time/prep	30 min/4 -6 preps

2.3 Remarks regarding sample quality and preparation

All kinds of biological fluids or semi-fluid samples can be processed e.g. serum, urine, or BAL. For successful nucleic acid purification, it is important to obtain a homogeneous, clear and non-viscous sample before loading onto the corresponding **RNA Virus Columns**. Therefore, check all samples (especially old or frozen ones) for precipitates. Avoid clearing samples by centrifugation/filtration before the RAV1-lysis step, because viruses may be associated with particles or aggregates. Incubation with Buffer RAV1 may be prolonged to dissolve and digest residual cell structures, precipitates and virus particles. However, RNA is sensitive to autolysis and prolonged incubation may cause degradation and decreased yields.

2.4 Remarks regarding elution

- Pure nucleic acids are finally eluted under low ionic strength conditions with RNase-free H_2O (pH about 7 -8) or slightly alkaline Buffer RE (5 mM Tris-HCl, pH 8.5).
- Elution can be performed in a single step with water/elution buffer as indicated in the protocol, obtaining at least 80% of the bound nucleic acids. To improve sensitivity, this elute can be used in a second elution step increasing the efficiency of elution and concentration of viral nucleic acids slightly. Alternatively, a second elution step can be performed with an additional volume of water/elution buffer releasing practically all bound nucleic acids but resulting in a lower concentrated, combined elute.
- RNA should be eluted with the water supplied and DNA with Elution Buffer RE. Buffer RE provides better storage conditions for DNA. To elute both types of nucleic acids together, use the pH proofed (pH 6 -8), RNase-free H_2O preheated to 70°C.

2.5 Remarks regarding quality control

- Buffers and **RNA Virus 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.

3 Storage conditions and preparation of working solutions

Attention:
Buffers RAV1 and RAW contain guanidine salts! 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 RAV1. 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 use, add 1 ml **Lysis Buffer RAV1** to the Carrier RNA tube. Dissolve the RNA and transfer it back to the RAV1 bottle.

Storage of Carrier RNA in Buffer RAV1:

- **Lysis Buffer RAV1 including Carrier RNA** can be stored at **room temperature** for **1-2 weeks**. Storage at room temperature prevents salt precipitation.
- **Lysis Buffer RAV1 including Carrier RNA** can 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 preheated at 40-60°C for a maximum of 5 min in order to redissolve salts.
- **Do not warm Buffer RAV1 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.

Before starting RNA isolation protocol prepare the following:

- **Wash Buffer RAV3:** Add the indicated volume of ethanol (96-100%) to Wash Buffer RAV3 Concentrate. Mark the label of the bottle to indicate that the ethanol is added. Store Wash Buffer RA3 at room temperature (20 -25°C) for up to one year.

	GeneProof PathogenFree RNA Isolation Kit	
Cat. No.	**50 preps** **IRNA050**	**250 preps** **IRNA250**
Wash Buffer RAV3 (Concentrate)	12.5 ml Add 50 ml ethanol	3 x 25 ml Add 100 ml ethanol to each vial

4 Safety instructions – risk and safety phrases

The following components of the **GeneProof PathogenFree RNA Isolation Kits** contain hazardous contents.

Wear gloves and goggles and follow the safety instructions given in this section.

Component	Hazard contents	Hazard symbol	Risk phases		Safety phrases
RAV1	Guanidine thiocyanate	Xn*	Harmful by inhalation, in contact with the skin and if swallowed	R 20/21/22	S 13
RAW	Guanidine hydrochloride + ethanol < 50%	Xn*	Flammable - Harmful if swallowed -Irritating to eyes and skin	R 10-22 36/38	S 7-16

Risk phrases

R 10	Flammable
R 20/21/22	Harmful by inhalation, in contact with the skin and if swallowed
R 22	Harmful if swallowed
R 36/38	Irritating to eyes and skin

Safety phrases

S 7	Keep container tightly closed.
S 13	Keep away from food, drink, and animal feedstuffs.
S 16	Keep away from sources of ignition - No Smoking!

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* Hazard labeling not neccessary if quantity per bottle below 125 g or ml (certificate of exemption according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 20 (3) and TRGS 200 7.1). For further information see Material Safety Data Sheet.

5 Viral RNA isolation from cell-free biological fluids

Before starting the preparation:

- Check if Wash Buffer RAV3 was prepared according to section 3.
- Preheat an aliquot of Elution Buffer RE/RNase-free H_2O to 70°C.

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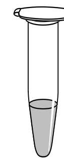
1 Lysis of viruses

Add 600 µl Buffer RAV1 containing Carrier RNA to 150 µl of the sample. Pipette mixture up and down and vortex well.

Incubate for 5 min at 70°C.

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 and to prevent clogging of the RNA Virus Columns). Take off the supernatant and proceed with step 2.*



150 µl sample

+ 600 µl RAV1

70°C
5 min

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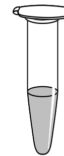
2 Adjust binding conditions

Add 600 µl ethanol (96 - 100%) to the clear lysis solution and mix by vortexing (10-15 s).



+ 600 µl
ethanol
mix

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3 Bind viral RNA

Place RNA Virus Columns in Collection Tubes (2 ml) and load 700 µl of lysed sample.

Centrifuge for 1 min at 8,000 x *g*.

The use of new Collection Tubes (2 ml) is recommended if infectious material has to be processed.

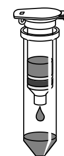
Load the residual lysis solution onto the RNA Virus Column.

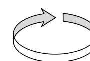
Centrifuge for 1 min at 8,000 x *g*.

Discard the Collection tube with the flow-through and place the RNA Virus Column into a new Collection Tube (2 ml).

More than two loading steps are not recommended.

load sample
stepwise





1 min
8,000 × g

4 Wash and dry silica membrane

1st wash

Add 500 µl Buffer RAW to the RNA Virus Column.

Centrifuge for 1 min at 8,000 x *g*.

Discard Collection Tube with the flow-through.

This washing step removes contaminants and PCR inhibitors.

+ 500 µl RAW

1 min
8,000 x g

2nd wash

Place the RNA Virus Column in a new Collection Tube (2 ml), add 600 µl Buffer RAV3 to the RNA Virus Column.

Centrifuge for 1 min at 8,000 x *g*.

Discard Collection Tube with the flow-through.

+ 600 µl RAV3

1 min
8,000 x g

3rd wash

Place the RNA Virus Column in a new Collection Tube (2 ml) and add 200 µl Buffer RAV3.

Centrifuge for 2-5 min at 11,000 x *g* to remove ethanolic Buffer RAV3 completely.

Discard Collection Tube with the flow-through.

+ 200 µl RAV3

2-5 min
11,000 x g

5 Dry silica membrane

Place the RNA Virus column into a new Collection Tube (2ml). Centrifuge for 1 min at 11,000 x g.

Or alternatively, incubate the RNA Virus Columns for 1 min at 70°C to remove any remaining traces of ethanol.

1 min

11,000 x g

6 Elute viral RNA

Place the RNA Virus Column into a new, sterile 1.5 ml microcentrifuge tube (not provided).

Add 50 µl RNase-free H_2O (preheated to 70°C) and incubate for 1-2 min.

Centrifuge for 1 min at 11,000 x *g*.

+ 50 µl RNase-free H_2O (70°C)

RT
1-2 min

1 min
11,000 x g

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
Small amounts or no viral nucleic acids in the eluate	*Problems with Carrier RNA* - Carrier RNA not added. - See remarks concerning storage of Buffer RAV1 with Carrier RNA (section 3). *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 RAV1, Carrier RNA and Elution Buffer RE.
Problems with sub-sequent 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 carry-over* - Prolong centrifugation steps in order to remove Buffer RAV3 completely.
General problems	*Clogged membrane* - Centrifuge plasma lysate before the addition of ethanol and subsequent loading onto the corresponding RNA Virus Columns

6.2 Ordering information

	Cat. No.	Package
PathogenFree RNA Isolation Kit	IRNA050	50 isolations
PathogenFree RNA Isolation Kit	IRNA250	250 isolations

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