

For general laboratory use.



High Pure Viral Nucleic Acid Kit

 **Version: 19**

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For isolation of viral nucleic acids for PCR or RT-PCR.

Cat. No. 11 858 874 001

1 kit
100 isolations

Store the kit at +15 to +25°C

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1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Contents / Function
1	green	Binding Buffer	<ul style="list-style-type: none"> 2 × 25 ml [6 M guanidine-HCl, 10 mM Tris-HCl, 10 mM urea, 20% Triton X-100 (w/v), pH 4.4 (+25°C)]
2		Poly(A)	<ul style="list-style-type: none"> Lyophilizate 2 mg poly(A) carrier RNA For binding of RNA
3	pink	Proteinase K	<ul style="list-style-type: none"> Lyophilizate 100 mg For the digestion of proteins
4 a	black	Inhibitor Removal Buffer	<ul style="list-style-type: none"> 33 ml, add 20 ml absolute ethanol [5 M guanidine-HCl, 20 mM Tris-HCl, pH 6.6 (+25°C) final concentration after addition of ethanol]
4 b	blue	Wash Buffer	<ul style="list-style-type: none"> 2 × 10 ml add 40 ml absolute ethanol each [20 mM NaCl, 2 mM Tris-HCl, pH 7.5 (+25°C) final concentrations after addition of ethanol]
5	colorless	Elution Buffer	<ul style="list-style-type: none"> 30 ml Water, PCR Grade
6		High Pure Filter Tubes	<ul style="list-style-type: none"> Two bags with 50 polypropylene tubes with two layers of glass fiber fleece, for use of up to 700 µl sample volume.
7		Collection Tubes	<ul style="list-style-type: none"> Eight bags with 50 polypropylene tubes (2 ml).

⚠ All solutions are clear, except Vial 1 Binding Buffer is clear to slightly turbid, and colorless to slightly yellowish viscous solution and should not be used if precipitates are present. Warm the solutions at +15 to +25°C or in a +37°C waterbath until the precipitates are dissolved.

1.2. Storage and Stability

Storage Conditions (Product)

Kit components are stable at +15 to +25°C until the expiration date printed on the label.

⚠ Improper storage at +2 to +8°C (refrigerator) or –15 to –25°C (freezer) will adversely impact nucleic acid purification when precipitates form in the solutions and may result in reduced binding efficiency.

Storage Conditions (Working Solution)

Solution	Storage
Proteinase K solution	–15 to –25°C
Poly(A) carrier RNA solution	–15 to –25°C

1.3. Additional Equipment and Reagents Required

- Absolute ethanol
- Standard tabletop microcentrifuge capable of 13,000 x g centrifugal force (e.g., Eppendorf 5415C or equivalent)
- Microcentrifuge tubes, 1.5 ml, sterile
- Optional: High Pure Viral Nucleic Acid Buffer Set*

1. General Information

1.4. Application

The High Pure Viral Nucleic Acid Kit is designed for the purification of viral nucleic acids from mammalian serum, plasma or whole blood.

When using whole blood total nucleic acids are purified including viral nucleic acids. The purified viral nucleic acids are applied in PCR or RT-PCR directly after elution in water, PCR grade.

1.5. Preparation Time

Total time	Approximately 20 min
Hands-on time	<10 min

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Purification of viral nucleic acids from 200 µl

- serum
- plasma
- whole blood

⚠ Samples containing precipitates must be centrifuged before purification.

Control Reactions

⚠ It is the user's own responsibility to apply an appropriate control concept.

General Considerations

Handling requirements

⚠ Binding Buffer and Inhibitor Removal Buffer contain guanidine hydrochloride which is an irritant. Always wear gloves and follow standard safety precautions to minimize contact when handling.

⚠ Never store or use the Binding Buffer and Inhibitor Removal Buffer near human or animal food.

⚠ Avoid contact of the Binding Buffer and Inhibitor Removal Buffer with the skin, eyes, or mucous membranes. If contact does occur, immediately wash the affected area with large amount of water. Burns can occur if left untreated. If the reagent spills, dilute with water before wiping dry.

⚠ Do not use any modified ethanol.

⚠ Do not pool reagents from different lots or from different bottles of the same lot.

⚠ Immediately after usage, close all bottles in order to avoid leakage, varying buffer concentrations or buffer conditions. After first opening store all bottles in an upright position.

Safety Information

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis/Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Do not contaminate the reagents with bacteria, virus, or nucleases. Use disposable pipets and nuclease free pipet tips only, to remove aliquots from reagent bottles. Use the general precautions described in the literature.
- Wash hands thoroughly after handling samples and reagents.
- Complete each phase of the PCR/RT-PCR workflow before proceeding to the next phase. For example, you should finish PCR/RT-PCR sample preparation before starting PCR/RT-PCR set-up. Sample preparation, PCR/RT-PCR set-up and the PCR/RT-PCR run itself should also be performed in separate locations.

Waste handling

- Dispose of unused reagents and waste should occur in accordance with country, federal state and local regulations.
- Please follow the instructions in the Safety Data Sheets (SDS).

2. How to Use this Product

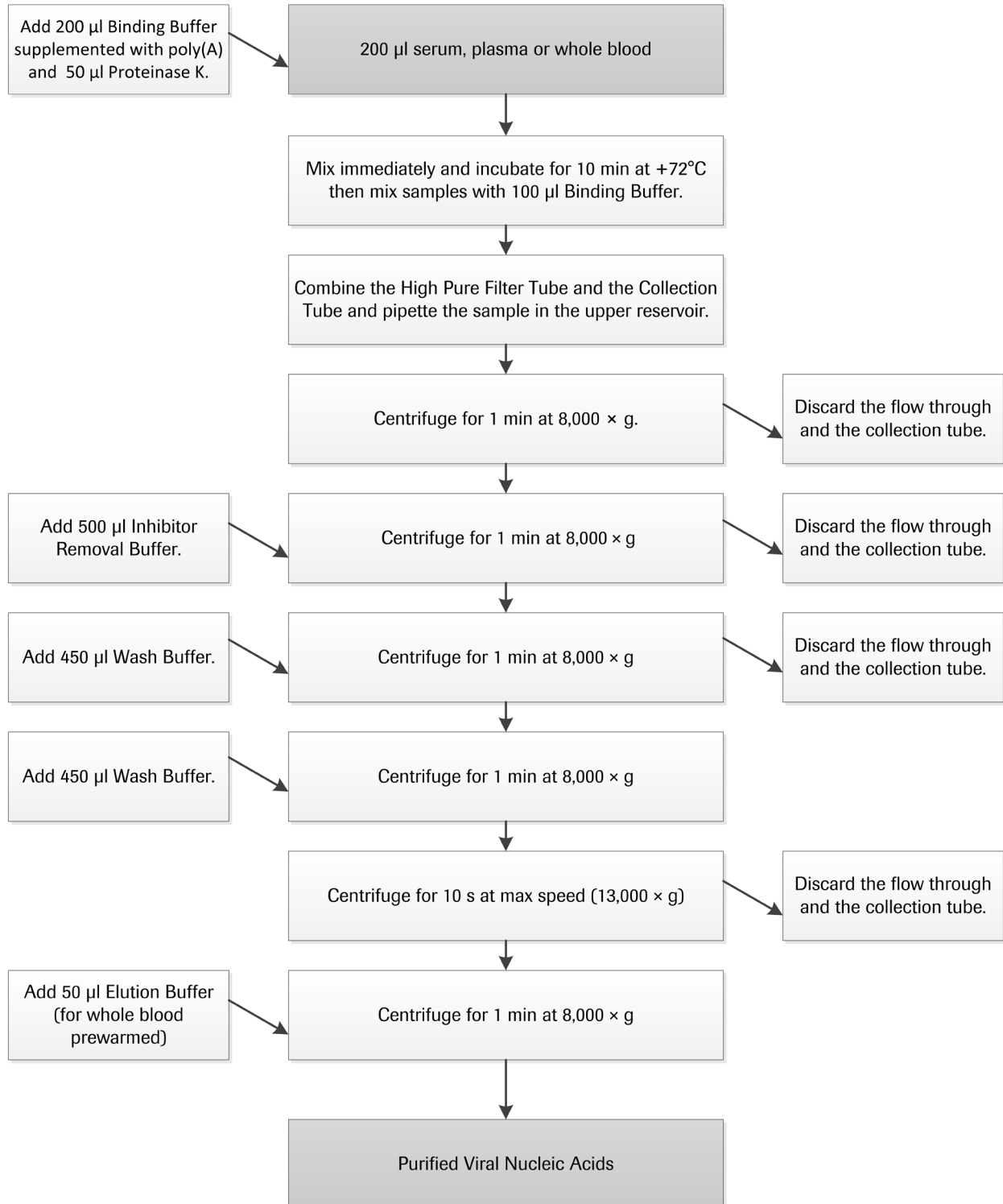
Working Solution

Beside the ready-to-use solutions supplied with this kit, you will need to prepare the following working solutions:

Content	Reconstitution / Preparation	Storage / Stability	For use in...
Proteinase K (Vial 3; pink cap)	<ul style="list-style-type: none"> Dissolve Proteinase K in 5 ml Elution Buffer and mix thoroughly. 	<ul style="list-style-type: none"> Store aliquots at –15 to –25°C. Stable for 12 months. 	Protocol Step 1: Cell lysis
Poly(A) carrier RNA (Vial 2)	<ul style="list-style-type: none"> Dissolve poly(A) carrier RNA (Vial 2) in 0.5 ml Elution Buffer (Vial 5). Prepare 50 µl aliquots. <p>Working solution:</p> <ul style="list-style-type: none"> Thaw one vial with 50 µl poly(A) carrier RNA and mix thoroughly with 2.5 ml Binding Buffer (Vial 1; green cap). 	<ul style="list-style-type: none"> Store aliquots at –15 to –25°C. <p>⚠ Prepare always fresh before use! Do not store!</p>	For the preparation of the working solution. Protocol step 1
Inhibitor Removal Buffer (Vial 4a; black cap)	<ul style="list-style-type: none"> Add 20 ml absolute ethanol to Inhibitor Removal Buffer and mix well. <p>i Label and date bottle accordingly after adding ethanol.</p>	<ul style="list-style-type: none"> Store at +15 to +25°C. Stable until the expiration date printed on kit label. 	Protocol step 6: To remove PCR inhibitors.
Wash Buffer (Vial 4; blue cap)	<ul style="list-style-type: none"> Add 40 ml absolute ethanol to each Wash Buffer and mix well. <p>i Label and date bottle accordingly after adding ethanol.</p>	<ul style="list-style-type: none"> Store at +15 to +25°C. Stable until the expiration date printed on kit label. 	Protocol step 8 and 9: Removal of residual impurities.

2.2. Protocols

Flow chart



2. How to Use this Product

Procedure for preparing nucleic acids from 200 µl samples of serum, plasma or whole blood.

i If larger sample volumes are to be used increase all components accordingly and load to the Filter Tubes multiple times.

Additional reagents can be taken from the High Pure Viral Nucleic Acid Buffer Set*.

⚠ For isolation of nucleic acids from whole blood use prewarmed Elution Buffer (+70°C).

- 1** To a nuclease free 1.5 ml microcentrifuge tube
Add 200 µl serum, plasma or whole blood
Add 200 µl working solution, freshly prepared, [carrier RNA-supplemented Binding Buffer]
Add 50 µl Proteinase K solution, and mix immediately.
Incubate for 10 min at +72°C.

- 2** Add 100 µl Binding Buffer and mix

- 3** To transfer the sample to a High Pure Filter Tube:
Insert one High Pure Filter Tube in one Collection Tube.
Pipet entire sample into the upper reservoir of the Filter Tube.

- 4** Insert the entire High Pure Filter Tube assembly into a standard tabletop centrifuge.
Centrifuge 1 min at 8,000 × g.

- 5** After centrifugation:
Remove the Filter Tube from the Collection Tube, discard the flowthrough liquid, and the Collection Tube.
Combine the Filter Tube with a new Collection Tube.

- 6** After combining the Filter Tube with a new Collection Tube.
Add 500 µl Inhibitor Removal Buffer to the upper reservoir of the Filter Tube.
Centrifuge 1 min at 8,000 × g.

- 7** After centrifugation:
Remove the Filter Tube from the Collection Tube, discard the flowthrough liquid, and the Collection Tube.
Combine the Filter Tube with a new Collection Tube.

- 8** After removal of inhibitors:
Add 450 µl Wash Buffer to the upper reservoir of the Filter Tube.
Centrifuge 1 min at 8,000 × g and discard the flowthrough

- 9** After the first wash and centrifugation:
Remove the Filter Tube from the Collection Tube, discard the flowthrough liquid, and the Collection Tube.
Combine the Filter Tube with a new Collection Tube.
Add 450 µl Wash Buffer to the upper reservoir of the Filter Tube.
Centrifuge 1 min at 8,000 × g and discard the flowthrough.
Leave the Filter Tube-Collection Tube assembly in the centrifuge and spin it for 10 sec at maximum speed (approximately 13,000 × g) to remove any residual Wash Buffer.
i The extra centrifugation time ensures removal of residual Wash Buffer.

- 10** Discard the Collection Tube and insert the Filter Tube into a nuclease free, sterile 1.5 ml microcentrifuge tube.

- 11** To elute the viral nucleic acids:
Add 50 µl Elution Buffer to the upper reservoir of the Filter Tube.
Centrifuge the tube assembly for 1 min at 8,000 × g.

- 12** The microcentrifuge tube now contains the eluted viral nucleic acids.
i Either use the eluted nucleic acids directly in PCR (10 to 20 µl DNA eluate) or RT-PCR (3.5 µl viral RNA) or store the eluted viral RNA at -80°C or the viral DNA at +2 to +8°C or at -15 to -25°C for later analysis.

3. Results

Each preparation was used as a template in PCR (DNA) or RT-PCR (RNA). All these templates produced highly specific PCR products showing the expected yields.

Testing of the High Pure Viral Nucleic Acid Kit has been accomplished with DNA Virus (HBV, CMV) and RNA Virus (HCV, HGV, HIV) positive human samples and has shown the expected results for specificity and sensitivity in PCR, RT-PCR analysis.

4. Troubleshooting

Observation	Possible cause	Recommendation
Low nucleic acid yield or purity	Kit stored under non-optimal conditions.	Store kit at +15 to +25°C at all times upon arrival.
	Buffers or other reagents were exposed to conditions that reduced their effectiveness.	Store all buffers at +15 to +25°C. Close all reagent bottles tightly after each use to preserve pH, stability, and freedom from contamination. After any lyophilized reagent is constituted, aliquot it and store the aliquot at –15 to –25°C.
	Ethanol not added to Wash Buffer and Inhibitory Removal Buffer	Add absolute ethanol to the buffers before using. After adding ethanol, mix the buffers well and store at +15 to +25°C. Always mark Wash Buffer vial and Inhibitory Removal Buffer vial to indicate whether ethanol has been added or not.
	Reagents and samples not completely mixed.	Always mix the sample tube well after addition of each reagent.
Poor elution of nucleic acids with water	Water has the wrong pH.	If you use your own water or buffer to elute nucleic acids from Filter Tube, be sure it has the same pH as the Elution Buffer supplied in the kit.
Absorbance ($A_{260\text{ nm}}$) reading of product too high	Glass fibers, which might coelute with nucleic acid, scatter light.	<ol style="list-style-type: none"> Remove High Pure Filter Tube from tube containing eluted sample and spin sample for 1 min at maximum speed. Transfer supernatant into a new tube without disturbing the glass fibers at the bottom of the original tube.
Low RNA yield	High levels of RNase activity.	Be careful to create an RNase-free working environment. Process starting material immediately or store it at –80°C until it can be processed. Use eluted RNA directly in downstream procedures or store it immediately at –80°C.
	Incomplete Proteinase K digestion.	Be sure to dissolve the lyophilized Proteinase K completely, as follows: <ol style="list-style-type: none"> Pipet 5 ml of Elution Buffer into the glass vial containing lyophilized Proteinase K. Stopper and invert the vial until all the lyophilizate (including any stuck to the rubber stopper) is dissolved. Aliquot the reconstituted enzyme, mark each aliquot with the date of reconstitution, and store at –15 to –25°C. <p>i <i>Reconstituted Proteinase K is stable for 12 months when stored properly.</i></p>

5. Additional Information on this Product

5.1. Test Principle

As a pre-requisite for the analysis of viral nucleic acids by the polymerase chain reaction (PCR) or RT-PCR the isolation of the analyte from serum, plasma or whole blood is required.

Virus lysis is accomplished by incubation of the sample in a special Lysis/Binding buffer in the presence of Proteinase K. Subsequently, nucleic acids bind specifically to the surface of glass fibers in the presence of a chaotropic salt (1). The binding reaction occurs within seconds due to the disruption of the organized structure of water molecules and the interaction of nucleic acids with the glass fibers surface. Thus, adsorption to the glass fiber fleece is favored. Since the binding process is specific for nucleic acids, the bound nucleic acids are purified from salts, proteins and other impurities by a washing step and are eluted in low salt buffer or water.

- ① Serum, plasma or whole blood are lysed by incubation with Binding buffer and Proteinase K.

- ② Nucleic acids are bound to the glass fibers pre-packed in the High Pure Filter Tube.

- ③ Bound nucleic acids are washed with a special Inhibitor Removal Buffer to get rid of PCR inhibitory contaminants. It allows even the application of heparinized sample material with > 100 U/ml heparin.

- ④ Washing of bound nucleic acids, purification from salts, proteins and other cellular impurities.

- ⑤ Purified nucleic acids are recovered using the Elution Buffer.









5.2. Quality Control

Series of MS2 RNA dilution are prepared, applied to the filter tubes, washed and eluted following the kit protocol. 3.5 µl of the eluate is analyzed by RT-PCR. The products are detected on agarose gel. At least 2×10^5 RNA molecules / 200 µl sample are guaranteed.

6. Supplementary Information

6.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
 Information Note: Additional information about the current topic or procedure.	
 Important Note: Information critical to the success of the current procedure or use of the product.	
   etc.	Stages in a process that usually occur in the order listed.
   etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

6.2. Changes to Previous Version

Layout changes
Editorial changes

6.3. Ordering Information

Roche offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our homepage lifescience.roche.com.

Product	Pack Size	Cat. No.
High Pure Viral Nucleic Acid Buffer Set	1 set, up to 100 isolations	12 011 875 001

6.4. Trademarks

HIGH PURE is a trademark of Roche.

All other product names and trademarks are the property of their respective owners.

6.5. License Disclaimer

For patent license limitations for individual products please refer to: <http://technical-support.roche.com>.

6.6. Regulatory Disclaimer

For general laboratory use.

6.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

6.8. Contact and Support

If you have questions or experience problems with this or any Roche product for Life Science, please contact our Technical Support staff. Our scientists are committed to providing rapid and effective help.

Please also contact us if you have suggestions for enhancing Roche product performance or using our products in new or specialized ways. Such customer information has repeatedly proven invaluable to the research community worldwide.

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support** Site.

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