For general laboratory use.
FOR *IN VITRO* USE ONLY.

High Pure Viral Nucleic Acid Kit

Version December 2008

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

Cat. No. 11 858 874 001 Kit for 100 isolations

Store the kit at +15 to +25°C

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PROTOCOL

1. What this Product Does

Number of Tests The kit is designed for 100 isolations.

Kit Contents

⚠ All solutions are clear, and should not be used when precipitates have formed. Warm the solutions at +15 to +25°C or in a 37°C waterbath until the precipitates have dissolved.

Vial/Cap	Label	Contents / Function
1 green	Binding Buffer	• 2 x 25 ml • [6 M guanidine-HCl, 10 mM Tris-HCl, 20% Triton® X-100 (w/v), pH 4.4 (25°C)].
2	Poly(A)	• Lyophilizate • 2 mg poly(A) carrier RNA • For binding of RNA
3 pink	Proteinase K	• Lyophilizate • 100 mg • For the digestion of proteins
4a black	Inhibitor Removal Buffer	• 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 blue	Wash Buffer	• 2 x 10 ml add 40 ml ethanol p.a each • [20 mM NaCl, 2 mM Tris-HCl, pH 7.5 (25°C) final concentrations after addition of ethanol]
5 colorless	Elution Buffer	• 30 ml • Nuclease-free, sterile, double distilled water
6	High Pure Filter Tubes	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	Eight bags with 50 polypropylene tubes (2 ml).

Storage and Stability

The High Pure Viral Nucleic Acid Kit components must be stored at +15 to +25°C. If properly stored, all kit components are stable through the expiration date printed on the label.

⚠ Please note, that 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.

Therefore, High Pure isolation kits are always shipped at +15 to +25°C.

Reconstituted poly(A) carrier RNA solution has to be stored in aliquots. Aliquots stored at –15 to –25°C are stable for 12 month.

Additional Equipment and Reagents Required

- Absolute ethanol
- Standard tabletop microcentrifuge capable of 13,000 × *g* centrifugal force (*e.g.,* Eppendorf 5415C or equivalent)
- Microcentrifuge tubes, 1.5 ml, sterile

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 nuclease-free water.

Preparation Time

Total time	Approx. 20 min
Hands-on time	<10 min

2. How To Use this Product

2.1 Before You Begin

Precautions

⚠ 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.

- Do not let these buffers touch your skin, eyes, or mucous membranes. If contact does occur, wash the affected area immediately with large amounts of water; otherwise, the reagent may cause burns. If you spill the reagent, dilute the spill with water before wiping it up.
- Never store or use the Binding Buffer near human or animal food.
- Always wear gloves and follow standard safety precautions when handling these buffers.

Handling Requirements

- Exercise the normal precautions required for handling all laboratory reagents.
- 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.
- Do not use a kit after its expiration date.
- Avoid contact of the Binding Buffer and Inhibitor Removal Buffer with the skin, eyes, or mucous membranes. If contact does occur, immediately wash 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.
- Use only calibrated pipettes.

Laboratory Procedures

- All human sourced material and all resulting waste should be considered potentially infectious. Thoroughly clean and disinfect all work surfaces with disinfectants recommended by the local authorities.
- 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 specimens and kit reagents.
- Avoid microbial and nuclease contamination of reagents when removing aliquots from reagent bottles.
- The use of sterile disposable pipettes is recommended.
- Wash hands thoroughly after handling samples and test reagents.

Waste handling

- Dispose of unused reagents and waste should occur in accordance with country, federal state and local regulations.
- Material Safety Data Sheets (MSDS) are available upon request from the local Roche office.

Sample Material

Purification of viral nucleic acids from 200 µl

- serum
- plasma
- whole blood

⚠ Samples containing precipitates must be centrifuged before purification.

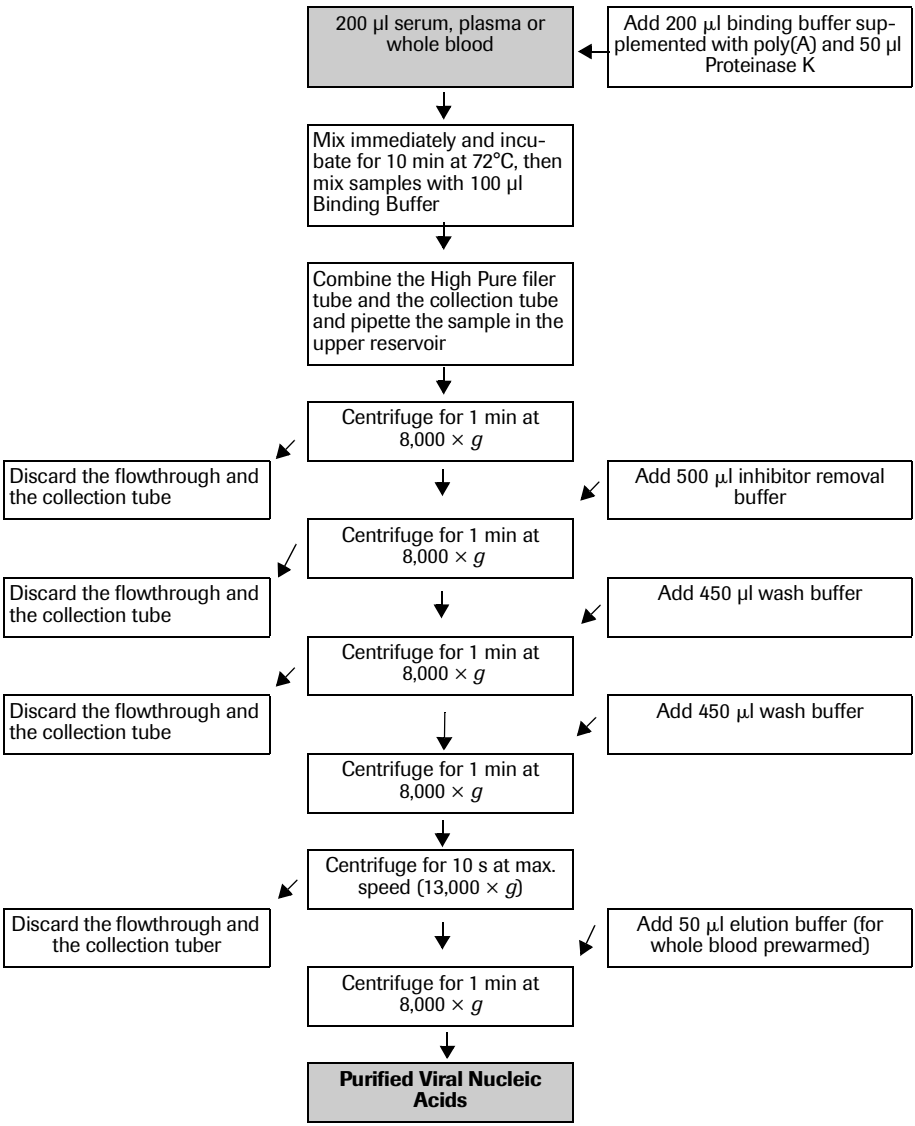
Preparation of Working Solutions

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

Content	Reconstitution/ Preparation	Storage and Stability	For use in
Proteinase K (Vial 3; pink cap)	Dissolve Proteinase K in 5 ml Elution Buffer and mix thouroghly.	Store aliquots at –15 to –25°C, stable for 12 month	Protocol Step 1: Cell lysis
poly(A) carrier RNA (Vial 2)	Dissolve poly(A) carrier RNA (vial 2) in 0.5 ml Elution Buffer (vial 5). Prepare 50 µl aliquots.	Store at –15 to –25°C.	For the preparation of the working solution
	Working solution: Thaw one vial with 50 µl poly(A) carrier RNA and mix thoroughly with 2.5 ml Binding Buffer (vial 1)	⚠Prepare always fresh before use! Do not store!	Protocol step 1
Inhibitor Removal Buffer (Vial 4a; black cap)	Add 20 ml absolute ethanol to Inhibitor Removal Buffer and mix well. ⚠ Label and date bottle accordingly after adding ethanol.	Store at +15 to +25°C. Stable through the expiration date printed on kit label	Protocol step 6: To remove PCR inhibitors
Wash Buffer (Vial 4; blue cap)	Add 40 ml absolute ethanol to each Wash Buffer and mix well. ⚠Label and date bottle accordingly after adding ethanol.	Store at +15 to +25°C. Stable through the expiration date printed on kit label.	Protocol step 8 and 9: Removal of residual impurities

Controls

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


200 µl serum, plasma or whole blood
Add 200 µl binding buffer supplemented with poly(A) and 50 µl Proteinase K
Mix immediately and incubate for 10 min at 72°C, then mix samples with 100 µl Binding Buffer
Combine the High Pure filer tube and the collection tube and pipette the sample in the upper reservoir
Centrifuge for 1 min at 8,000 × g
Discard the flowthrough and the collection tube
Add 500 µl inhibitor removal buffer
Centrifuge for 1 min at 8,000 × g
Discard the flowthrough and the collection tube
Add 450 µl wash buffer
Centrifuge for 1 min at 8,000 × g
Discard the flowthrough and the collection tube
Add 450 µl wash buffer
Centrifuge for 1 min at 8,000 × g
Centrifuge for 10 s at max. speed (13,000 × g)
Discard the flowthrough and the collection tuber
Add 50 µl elution buffer (for whole blood prewarmed)
Centrifuge for 1 min at 8,000 × g
Purified Viral Nucleic Acids

Procedure for Preparing Nucleic Acids from 200 µl Samples of Serum, Plasma or Whole Blood.

If larger sample volumes (up to 300 µl) are to be used increase all components accordingly and load to the Filter Tubes multiple times

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

❶	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.
❷	Add 100 µl Binding Buffer and mix.
❸	To transfer the sample to a High Filter Tube: • Insert one High Filter Tube in one Collection Tube. • Pipet entire sample into the upper reservoir of the Filter Tube.
❹	• Insert the entire High Pure Filter Tube assembly into a standard tabletop centrifuge. • Centrifuge 1 min at 8,000 × *g*.
❺	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.
❻	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*.
❼	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.
❽	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.

❾ 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 s at maximum speed (approx. 13,000 × *g*) to remove any residual Wash Buffer.

The extra centrifugation time ensures removal of residual Wash Buffer.

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

⓫ 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*.

⓬ The microcentrifuge tube now contains the eluted viral nucleic acids.

Either use the eluted nucleic acids directly in PCR (10 – 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

Experimental Results

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

Typical Results

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

	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 ($A_{260\ nm}$) reading of product too high	Glass fibers, which might coelute with nucleic acid, scatter light	1. Remove High Pure Filter Tube from tube containing eluted sample and spin sample for 1 min at maximum speed. 2. 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.
	Carrier RNA not completely dissolved	Poly(A) Carrier RNA, if dissolved in Binding Buffer, will precipitate when stored. Thus, be careful to prepare the Binding Buffer supplemented with Carrier RNA solution exactly as outlined in the preparation of working solutions.

	Possible Cause	Recommendation
Low RNA yield	Incomplete Proteinase K digestion	Be sure to dissolve the lyophilized Proteinase K completely, as follows: 1. Pipet 5 ml of Elution Buffer into the glass vial containing lyophilized Proteinase K. 2. Stopper and invert the vial until all the lyophilizate (including any stuck to the rubber stopper) is dissolved. 3. Aliquot the reconstituted enzyme, mark each aliquot with the date of reconstitution, and store at –15 to –25° C. ⚠ Reconstituted Proteinase K is stable for 12 months when stored properly.

5. Additional Information on this Product

How this Product Works

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.

Test principle

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.

References

1 Vogelstein B *et al.* (1979) Preparative and analytical purification of DNA from agarose. *Proc Natl Acad Sci USA* **76** (2), 615-619.
2 Mackay IM *et al.* (2003) Molecular Assays for Detection of Human Metapneumovirus. *Journal of Clinical Microbiology* **41**(1),100-105.
3 Greenberger S *et al.* (2004) Transcription-controlled gene therapy against tumor angiogenesis. *J Clin Invest.* **113** (7),1017–1024.
4 Koidl C *et al.* (2004) Detection of transfusion transmitted virus DNA by real-time PCR. *Journal of Clinical Virology* **29**, 277–281.
5 Widtschwendter A *et al.* (2004) Analysis of Aberrant DNA Methylation and Human Papillomavirus DNA in Cervicovaginal Specimens to Detect Invasive Cervical Cancer and Its Precursors. *Clinical Cancer Research* **10**, 3396-3400.

Quality Control

Series of MS 2 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. Typical experiments show a sensitivity level of around 350 RNA molecules/PCR reaction.

6. Supplementary Information

6.1 Conventions

Text Conventions To make information consistent and memorable, the following text conventions are used in this Instruction Manual:

Text Convention	Usage
Numbered stages labeled ①, ②, etc☆	Stages in a process that usually occur in the order listed
Numbered instructions labeled ❶, ❷, etc☆	Steps in a procedure that must be performed in the order listed
Asterisk *	Denotes a product available from Roche Applied Science.

Symbols In this Instruction Manual, the following symbols are used to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.
	Important Note: Information critical to the success of the procedure or use of the product.

6.2 Changes to Previous Version

New important note (Section: Before you begin)

6.3 Ordering Information

Roche Applied Science 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 home page, www.roche-applied-science.com, and our Special Interest Sites including:

- Nucleic Acid Isolation and Purification: http://www.roche-applied-science.com/napure
- PCR - Innovative Tools for Amplification: http://www.roche-applied-science.com/pcr

	Product	Pack Size	Cat. No.
Associated Kits	High Pure 16 System Viral Nucleic Acid Kit	96 reactions	12 011 816 001
	High Pure Viral Nucleic Acid Buffer Set	100 reactions	12 011 875 001
	High Pure PCR Template Preparation Kit	100 purifications	11 796 828 001
	High Pure Viral RNA Kit	100 purifications	11 858 882 001
LightCycler® Kits for PCR	LightCycler® DNA Master HybProbe	1 kit (96 reactions) 1 kit (480 reactions)	12 015 102 001 12 158 825 001
	LightCycler® FastStart DNA Master HybProbe	1 kit (96 reactions) 1 kit (480 reactions)	03 003 248 001 12 239 272 001
	LightCycler® FastStart DNA Master^{PLUS} Hyb-Probe	1 kit (96 reactions) 1 kit (480 reactions)	03 515 575 001 03 515 567 001
	LightCycler® FastStart DNA Master^{PLUS} Hyb-Probe, 100 µl Reactions	1 kit (384 reactions)	03 752 178 001
	LightCycler® DNA Master SYBR Green I	1 kit (96 reactions) 1 kit (480 reactions)	12 015 099 001 12 158 817 001
	LightCycler® FastStart DNA Master SYBR Green I	1 kit (96 reactions) 1 kit (480 reactions)	03 003 230 001 12 239 264 001
	LightCycler® FastStart DNA Master^{PLUS} SYBR Green I	1 kit (96 reactions) 1 kit (480 reactions)	03 515 869 001 03 515 885 001
	LightCycler® FastStart DNA Master^{PLUS} SYBR Green I, 100 µl Reactions	1 kit (384 reactions)	03 752 186 001
LightCycler® Kits for RT-PCR	LightCycler® RNA Master HybProbe	1 kit (96 reactions)	03 018 954 001
	LightCycler® RNA Master SYBR Green I	1 kit (96 reactions)	03 064 760 001
	LightCycler® RNA Amplification Kit HybProbe	1 kit (96 reactions)	12 015 145 001
	LightCycler® RNA Amplification Kit SYBR Green I	1 kit (96 reactions)	12 015 137 001
	LightCycler® Control Kit RNA	1 kit (50 reactions)	12 158 841 001
	High Pure PCR Product Purification Kit	50 purifications 250 purifications	11 732 668 001 11 732 676 001
Single reagents	Reverse Transcriptase, M-MuLV	500 U	11 062 603 001
	Reverse Transcriptase AMV	500 U 1,000 U	11 495 062 001 10 109 118 001

	Product	Pack Size	Cat. No.
Single reagents	Protector RNase Inhibitor	2000 U 10 000 U	03 335 399 001 03 335 402 001
	Transcriptor Reverse Transcriptase	250 U 500 U 2000 U	03 531 317 001 03 531 295 001 03 531 287 001
	Transcriptor First Strand cDNA Synthesis Kit	1 kit (50 reactions)	04 379 012 001

6.4 Trademarks

Contact and Support

If you have questions or experience problems with this or any Roche Applied Science (RAS) product, please contact our Technical Support staff. Our scientists commit themselves to providing rapid and effective help.
We also want you to contact us if you have suggestions for enhancing RAS product performance or using our products in new or specialized ways. Such customer information has repeatedly proven invaluable to RAS and the worldwide research community.

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

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Roche Diagnostics GmbH
Roche Applied Science
68298 Mannheim
Germany