For general laboratory use.

Print Protocol | Print Changes to Previous Version | Print Safety Precautions

High Pure Viral RNA Kit

Version 18

Content version: July 2011

For the isolation of viral RNA for RT-PCR

Cat. No. 11 858 882 001 Kit for up to 100 purifications

Store the kit at +15 to +25°C

www.roche-applied-science.com

Table of Contents

PROTOCOL

1. What this Product Does

Number of Tests The kit can be used for up to 100 purifications of viral RNA for RT-PCR.

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 × 25 ml ▪ 4.5 M guanidine-HCl, 50 mM Tris-HCl, 30% Triton X-100 (w/v), pH 6.6 (+25°C).
2	Poly(A)	▪ Lyophilizate ▪ 2 mg poly(A) carrier RNA ▪ For binding of RNA
3a 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
3 blue	Wash Buffer	▪ 2 × 10 ml, add 40 ml absolute ethanol to each vial Wash Buffer ▪ 20 mM NaCl, 2 mM Tris-HCl, pH 7.5 (+25°C) final concentrations after addition of ethanol
4 colorless	Elution Buffer	▪ 30 ml ▪ Water, PCR grade
5	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.
6	Collection Tubes	Eight bags with 50 polypropylene tubes (2 ml).

Storage and Stability

The High Pure Viral RNA Kit components must be stored at +15 to +25°C. If properly stored, all kit components are stable until 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 ambient temperature.

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 RNA Kit is designed to purify intact viral RNA from serum or plasma samples. Viral RNA is used for RT-PCR analysis directly after elution in PCR grade water.

⚠ RNA preparations obtained are suitable for RT-PCR; they are not tested for other applications.

Assay Time

Total time	Approximately 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 a chemical hazard and irritant.

- Avoid contact of the buffers with skin, eyes, or mucous membranes. If contact does occur, immediately wash with large amounts of water. Burns can occur if left untreated. If the reagent spills, dilute with water before wiping dry.
- Always store and use the buffers away from food for humans and animals.
- Always wear gloves, and follow standard safety precautions during handling.

Sample Material

- 200 – 600 µl research samples, such as serum, plasma, urine, or cell culture supernatant.

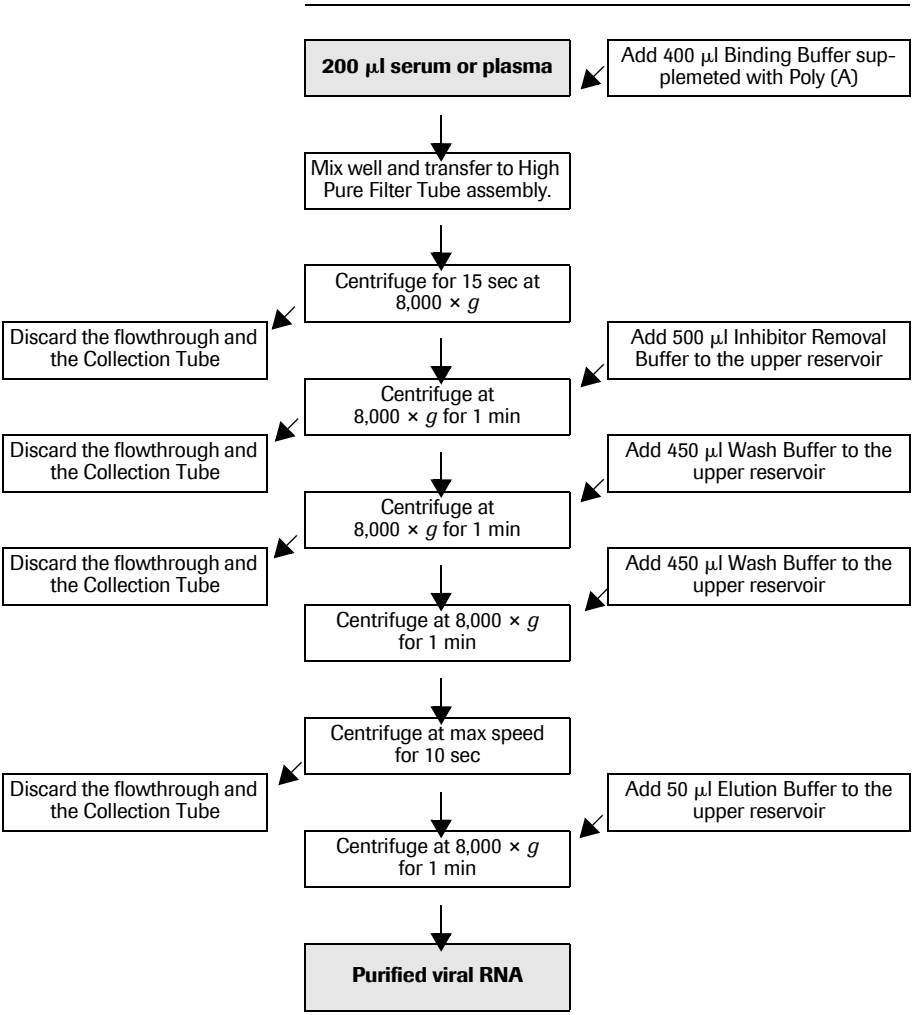
Preparation of Working Solutions

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
poly(A) carrier RNA (Vial 2)	Dissolve poly(A) carrier RNA (Vial 2) in 0.4 ml Elution Buffer (Vial 4). Prepare aliquots of 50 µl for running 8 × 12 purifications. Prepare aliquots of 100 µl for running 4 × 25 purifications.	Store at -15 to -25°C.	For the preparation of working solution
	Working solution: For 12 purifications, thaw one vial with 50 µl poly(A) carrier RNA and mix thoroughly with 5 ml Binding Buffer (vial 1). For 25 purifications, thaw one vial with 100 µl poly(A) carrier RNA and mix thoroughly with 10 ml Binding Buffer (Vial 1).	⚠ Prepare always fresh before use! Do not store!	Protocol Step 1
Inhibitor Removal Buffer (Vial 3a; 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 until the expiration date printed on kit label	Protocol Step 5: To remove PCR inhibitors
Wash Buffer (Vial 3; blue cap)	Add 40 ml absolute ethanol to each vial Wash Buffer before use and mix well. ⚠ Label and date bottle accordingly after adding ethanol.	Store at +15 to +25°C. Stable until the expiration date printed on kit label.	Protocol Step 6 and 7: Removal of residual impurities

Controls

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


200 µl serum or plasma
Add 400 µl Binding Buffer supplemeted with Poly (A)
Mix well and transfer to High Pure Filter Tube assembly.
Centrifuge for 15 sec at 8,000 × g
Discard the flowthrough and the Collection Tube
Add 500 µl Inhibitor Removal Buffer to the upper reservoir
Centrifuge at 8,000 × g for 1 min
Discard the flowthrough and the Collection Tube
Add 450 µl Wash Buffer to the upper reservoir
Centrifuge at 8,000 × g for 1 min
Discard the flowthrough and the Collection Tube
Add 450 µl Wash Buffer to the upper reservoir
Centrifuge at 8,000 × g for 1 min
Centrifuge at max speed for 10 sec
Discard the flowthrough and the Collection Tube
Add 50 µl Elution Buffer to the upper reservoir
Centrifuge at 8,000 × g for 1 min
Purified viral RNA

Protocol for Preparing Viral RNA from 200 µl Serum or Plasma Sample.

⚠ If larger sample volumes (up to 600 µl) are to be used increase all components accordingly and load to the Filter Tubes multiple times. The number of total reactions of the kit decreases when larger samples volumes are processed.

❶ To a nuclease-free 1.5 ml microcentrifuge tube:
- Add 200 µl serum or plasma.
- Add 400 µl Working solution [Carrier RNA supplemented Binding Buffer] and mix well.

🔍 The RNA yield can be increased twofold by an optional incubation step, thus resulting in higher sensitivity. After adding the Binding Buffer to the sample, simply incubate the mixture at +15 to +25°C for 10 min. This incubation step can be omitted when time to result is critical.

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

❸
- Insert the entire High Pure Filter Tube assembly into a standard tabletop centrifuge.
- Centrifuge the tube assembly 15 sec at 8,000 × *g*.

❹ After centrifugation:
- Remove the Filter Tube from the Collection Tube, discard the flowthrough liquid, and the Collection Tube.
- Insert the Filter Tube into a new Collection Tube.

❺ After re-inserting the Filter Tube:
- Add 500 µl Inhibitor Removal Buffer to the upper reservoir of the Filter Tube assembly and centrifuge 1 min at 8,000 × *g*.
- Discard flowthrough and combine Filter Tube with a new Collection Tube.

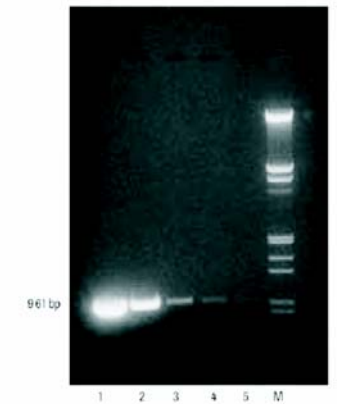
❻ After removal of inhibitors:
- Add 450 µl Wash Buffer to the upper reservoir of the Filter Tube.
- Centrifuge 1 min at 8,000 × *g* and discard the flowthrough.

❼ After the first wash and centrifugation:
- Remove the Filter Tube from the Collection Tube, discard the flowthrough liquid, and the Collection Tube.
- Insert the Filter Tube into a new Collection Tube.
- Add 450 µl Wash Buffer to the upper reservoir of the Filter Tube.
- Centrifuge 1 min at 8,000 × *g*.
- 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.

🔍 The extra centrifugation time ensures removal of residual Wash Buffer.

❽	Discard the Collection Tube and insert the Filter Tube into a clean, sterile 1.5 ml microcentrifuge tube.
❾	To elute the viral RNA: ▪ Add 50 µl Elution Buffer to the upper reservoir of the Filter Tube. ▪ Centrifuge the tube assembly for 1 min at 8,000 × *g*.
❿	The microcentrifuge tube now contains the eluted viral RNA. Either use the eluted RNA directly in RT-PCR or store the eluted RNA at –80°C for later analysis. Use 3.5 – 6 µl of the eluate for the reverse transcription reaction.

Serial dilutions of purified MS2 phage RNA were applied to the filter tubes, washed and eluted following the kit protocol. 3.5 µl of the 50 ml eluate were analyzed by two-step RT-PCR using primers that resulted in a fragment of 961 bp. The numbers of RNA molecules per PCR reaction indicated in the figure below correspond to the assumed quantitative recovery.



RT-PCR analysis of MS2 RNA isolated with the High Pure Viral RNA Kit.

Lane 1: 3.5 x 10^7 molecules/PCR;
Lane 2: 3.5 x 10^6 molecules/PCR;
Lane 3: 3.5 x 10^5 molecules/PCR
Lane 4: 3.5 x 10^4 molecules/PCR
Lane 5: 35 molecules/PCR
Lane M: DNA Molecular Weight Marker III

Furthermore, the kit was used to prepare genomic RNA from viruses [for example, hepatitis C virus (HCV), hepatitis G virus (HGV), and human immunodeficiency virus (HIV)] for research applications. Each preparation was used as a template in RT-PCR.

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.

5. Additional Information on this Product

How this Product Works

As a pre-requisite for the analysis of viral RNA by the reverse transcription polymerase chain reaction (RT-PCR) the isolation of the analyte from serum or plasma is required.

The High Pure Viral RNA Kit accomplishes virus lysis by incubation of the sample in a special Binding Buffer. Subsequently nucleic acids bind specifically to the surface of glass fibers in the presence of a chaotropic salt (guanidine-HCl; 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 finally eluted in low salt Elution Buffer or PCR grade water. The purified viral RNA is free of intact virus, nucleases, and all cellular components that interfere with RT-PCR and can be applied directly for RT-PCR. 50 µl eluate is sufficient for 8-14 RT-PCR reactions.

Included in the kit is a special Inhibitor Removal Buffer resulting in improved sensitivity and reproducibility of RT-PCR assays performed with nucleic acid templates isolated with this kit. Especially, the use of the Inhibitor Removal Buffer allows even the application of heparinized sample material containing >100 U/ml heparin.

The High Pure Viral RNA Kit

- saves time, because the kit does not require extraction with organic solutions or nucleic acid precipitation and thus can prepare multiple RT-PCR templates in approximately 10 minutes
- accommodates a wide variety of samples, because the same kit can purify viral RNA from several bodily fluids
- minimizes RNA loss, because the kit removes contaminants without precipitation or solvent extraction
- increases lab safety, because the kit minimizes the handling of potentially hazardous samples and does not use hazardous organic solvents

Basic steps

①	Serum or plasma are lysed by incubation with Binding Buffer.
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 RT-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 Mederle I. *et al.* (2003) Mucosal administration of three recombinant Mycobacterium bovis BCG–SIVmac251 strains to cynomolgus macaques induces rectal IgAs and boosts systemic cellular immune responses that are primed by intradermal vaccination. *Vaccine* **21**, 4153-4166

3 Peyrefitte C.M. *et al.* (2003) Evidence for in vitro falsely-primed cDNAs that prevent specific detection of virus negative strand RNAs in dengue-infected cells: improvement by tagged RT-PCR. *J. of Virol. Methods* **113**, 19-28

4 de Waal L. *et al.* (2004) Evaluation of BBG2Na in infant macaques: specific immune responses after vaccination and RSV challenge. *Vaccine* **22,** 915-922.

5 Munemura T *et al.* (2003) Genetic diversity of enterovirus 71 isolated from cases of hand, foot and mouth disease in Yokohama City between 1982 and 2000. *Arch Virol* **148**, 253–263

6 Zhao H *et al.* (2003) Recombinant Newcastle disease virus as a viral vector: effect of genomic location of foreign gene on gene expression and virus replication. *J Gen Virol* **84**, 781-788.

7 Prince AM *et al.* (2004) Hepatitis C virus replication kinetics in chimpanzees with self-limited and chronic infections. *Journal of Viral Hepatitis* **11**(3), 236-242.

8 Saijo M *et al.* (2004) Possible Horizontal Transmission of Crimean-Congo Hemorrhagic Fever Virus from a Mother to her Child. *Jpn. J. Infect. Disease* **57**, 55-57

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.

6. Supplementary Information

6.1 Conventions

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

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 document, 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 Versions

- Additional Working Solution preparation added
- Editorial changes

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 PCR Template Preparation Kit	100 purifications	11 796 828 001
	High Pure PCR Product Purification Kit	50 purifications 250 purifications	11 732 668 001 11 732 676 001
	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
	Transcriptor First Strand cDNA Synthesis Kit	1 kit (50 reactions) 1 kit (100 reactions) 1 kit (200 reactions)	04 379 012 001 04 896 866 001 04 897 030 001
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
	Protector RNase Inhibitor	2,000 U 10,000 U	03 335 399 001 03 335 402 001
	Transcriptor Reverse Transcriptase	250 U (25 reactions) 500 U (50 reactions) 2,000 U (200 reactions)	03 531 317 001 03 531 295 001 03 531 287 001
	Water, PCR Grade	25 ml (25 vials of 1 ml) 25 ml (1 vial of 25 ml) 100 ml (4 vials of 25 ml)	03 315 932 001 03 315 959 001 03 315 843 001

6.4 Trademarks

HIGH PURE, HYBPROBE and LIGHTCYCLER are trademarks of Roche.
SYBR is a registered trademark of Molecular Probes Inc.
All other product names and trademarks are the property of their respective owners.

6.5 Regulatory Disclaimer

For general laboratory use.

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 world-wide research community.

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

www.roche-applied-science.com/support

To call, write, fax, or email us, visit the Roche Applied Science home page, www.roche-applied-science.com, and select your home country.
Country-specific contact information will be displayed.
On the Roche Applied Science home page select **Printed Materials** to find:

- in-depth Technical Manuals
- Lab FAQS: Protocols and references for life science research
- our quarterly Biochemica Newsletter
- Material Safety Data Sheets
- Pack Inserts and Product Instructions

or to request hard copies of printed materials.

0812.**11879855001**®

Roche Diagnostics GmbH
Roche Applied Science
68298 Mannheim
Germany