For general laboratory use.

High Pure Viral RNA Kit

Version: 21
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For the isolation of viral RNA for RT-PCR.

Cat. No. 11 858 882 001	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	Function / description	Content
1	green	High Pure Viral RNA Kit, Binding Buffer	Contains 4.5 M guanidine-HCl, 50 mM Tris-HCl, 25% polidocanol (w/v).	2 bottles, 25 mL each
2	–	High Pure Viral RNA Kit, Poly (A)	▪ For binding of RNA. ▪ Lyophilized	1 vial, 2 mg Poly (A) carrier RNA
3a	black	High Pure Viral RNA Kit, Inhibitor Removal Buffer	Contains 5 M guanidine-HCl, 20 mM Tris-HCl, pH 6.6 (+25°C); final concentration after addition of ethanol. ℹ *See Section* **Working Solution** *for information on preparing the solution.*	1 bottle, 33 mL
3	blue	High Pure Viral RNA Kit, Wash Buffer	Contains 20 mM NaCl, 2 mM Tris-HCl, pH 7.5 (+25°C); final concentrations after addition of ethanol. ℹ *See Section* **Working Solution** *for information on preparing the solution.*	2 bottles, 10 mL each
4	colorless	High Pure Viral RNA Kit, Elution Buffer	Water, PCR grade	1 bottle, 30 mL
5	–	High Pure Viral RNA Kit, High Pure Filter Tubes	For use of up to 700 µL sample volume.	2 bags, 50 polypropylene filter tubes with two layers of glass fiber fleece each
6	–	High Pure Viral RNA Kit, Collection Tubes	For viral RNA isolation.	8 bags, 50 polypropylene tubes, 2 mL each

⚠ ***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 water bath until the precipitates have dissolved.***

ℹ *The buffers can show a slight yellow color. This will have no impact on the function of the buffer.*

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +15 to +25°C, the kit is stable through the expiry date printed on the label.

<table>
<tr><th>Vial / bottle</th><th>Cap</th><th>Label</th><th>Storage</th></tr>
<tr><td>1</td><td>green</td><td>Binding Buffer</td><td rowspan="5">Store at +15 to +25°C.
⚠ Storage at +2 to +8°C or −15 to −25°C will adversely impact nucleic acid isolation due to the formation of precipitates in the solutions and may result in reduced binding efficiency.</td></tr>
<tr><td>2</td><td>–</td><td>Poly (A)</td></tr>
<tr><td>3a</td><td>black</td><td>Inhibitor Removal Buffer</td></tr>
<tr><td>3</td><td>blue</td><td>Wash Buffer</td></tr>
<tr><td>4</td><td>colorless</td><td>Elution Buffer</td></tr>
<tr><td>5</td><td>–</td><td>High Pure Filter Tubes</td><td rowspan="2">Store at +15 to +25°C.</td></tr>
<tr><td>6</td><td>–</td><td>Collection Tubes</td></tr>
</table>

1.3. Additional Equipment and Reagent required

Standard laboratory equipment and reagents

- Absolute ethanol
- Standard tabletop microcentrifuge capable of 13,000 × *g* centrifugal force
- Sterile microcentrifuge tubes, 1.5 mL

1.4. Application

The High Pure Viral RNA Kit is designed to isolate 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.***

1.5. Preparation Time

Assay Time

Total time	Approximately 20 minutes.
Hands-on time	<10 minutes.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Isolation of viral RNA from 200 to 600 µL research samples, such as:

- Serum
- Plasma
- Urine
- Cell culture supernatant

Control Reactions

ℹ *It is the user's responsibility to implement an appropriate experiment control concept.*

General Considerations

Handling requirements and 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.***

- 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 a 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.
- Do not allow the Binding Buffer and Inhibitor Removal Buffer to mix with sodium hypochlorite found in commercial bleach solutions. This mixture can produce a highly toxic gas.

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.
- Use only calibrated pipettes.
- 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 pipettes and nuclease-free pipette tips only to remove aliquots from reagent bottles. Use the general precautions described in the literature.
- Wash hands thoroughly after handling samples and reagents.
- Finish 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 setup. Sample preparation, PCR/RT-PCR setup, and the PCR/RT-PCR run itself should also be performed in separate locations.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on documentation.roche.com, or upon request from the local Roche office.

Working Solution

Prepare the following working solutions:

Content	Reconstitution / preparation	Storage and stability	For use in...
Poly (A) (Vial 2)	Dissolve Poly (A) carrier RNA (Vial 2) in 0.4 mL Elution Buffer (Bottle 4). ▪ Prepare aliquots of 50 µL for running 8 × 12 isolations. ▪ Prepare aliquots of 100 µL for running 4 × 25 isolations.	▪ Store aliquots at −15 to −25°C. ▪ Stable for 12 months.	For the preparation of working solution.
	▪ For 12 isolations, thaw one vial of 50 µL Poly (A) carrier RNA and mix thoroughly with 5 mL Binding Buffer (Bottle 1). ▪ For 25 isolations, thaw one vial of 100 µL Poly (A) carrier RNA and mix thoroughly with 10 mL Binding Buffer (Bottle 1).	⚠ ***Always prepare fresh before use; do not store.***	Protocol, Step 1
Inhibitor Removal Buffer (Bottle 3a)	Add 20 mL absolute ethanol to Inhibitor Removal Buffer and mix well. ⚠ ***Label and date bottle after adding ethanol.***	▪ Store at +15 to +25°C. ▪ Stable through the expiry date printed on kit label.	Protocol, Step 5: To remove PCR inhibitors.
Wash Buffer (Bottle 3)	Add 40 mL absolute ethanol to each Wash Buffer and mix well. ⚠ ***Label and date bottle after adding ethanol.***	▪ Store at +15 to +25°C. ▪ Stable through expiry date printed on kit label.	Protocol, Steps 6 and 7: Removal of residual impurities.

2.2. Protocols

Experimental overview

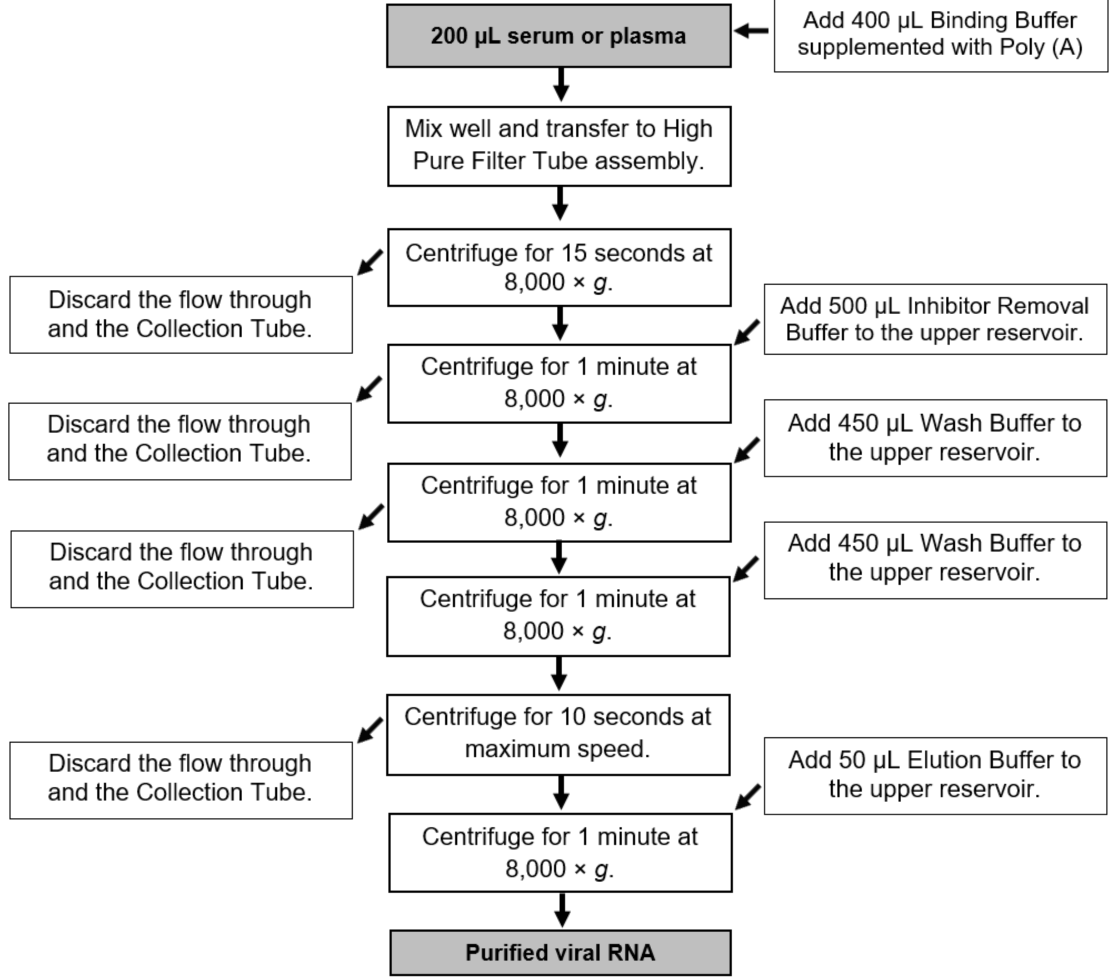

Fig. 1: Isolation of viral RNA from serum or plasma.

Isolation of viral RNA from 200 µL serum or plasma sample

ℹ *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.*

ℹ *See Section* **Working Solution** *for information on preparing solutions.*

1. To a nuclease-free 1.5 mL microcentrifuge tube, add:
   - 200 µL serum or plasma.
   - 400 µL Working solution (Carrier RNA supplemented Binding Buffer) and mix well.

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

2. To transfer the sample to a High Pure Filter Tube:
   - Insert one High Pure Filter Tube into one Collection Tube.
   - Pipette entire sample into the upper reservoir of the Filter Tube (maximum 700 µL).

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

4. After centrifugation:
   - Remove the Filter Tube from the Collection Tube; discard the flow through liquid and the Collection Tube.
   - Insert the Filter Tube into a new Collection Tube.

5. After re-inserting the Filter Tube:
   - Add 500 µL Inhibitor Removal Buffer to the upper reservoir of the Filter Tube assembly and centrifuge 1 minute at 8,000 × *g*.
   - Discard flow through and combine Filter Tube with a new Collection Tube.

6. After removal of inhibitors:
   - Add 450 µL Wash Buffer to the upper reservoir of the Filter Tube.
   - Centrifuge 1 minute at 8,000 × *g* and discard the flow through.

7. After the first wash and centrifugation:
   - Remove the Filter Tube from the Collection Tube; discard the flow through 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 minute at 8,000 × *g*.
   - Leave the Filter Tube-Collection Tube assembly in the centrifuge and spin it for 10 seconds at maximum speed (approximately 13,000 × *g*) to remove any residual Wash Buffer.

   ℹ *The extra centrifugation time ensures removal of residual Wash Buffer.*

8. Discard the Collection Tube and insert the Filter Tube into a clean, sterile 1.5 mL microcentrifuge tube.

9. To elute the viral RNA:
   - Add 50 µL Elution Buffer to the upper reservoir of the Filter Tube.
   - Centrifuge the tube assembly for 1 minute at 8,000 × *g*.

10. 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.

    ℹ *Use 3.5 to 6 µL of the eluate for the reverse transcription reaction.*

3. Results

Sample materials and conditions

Validation of the High Pure Viral RNA Kit is accomplished with RNA Virus HCV samples.
Human samples of Citrate Plasma were spiked with a dilution series of a virus stock solution prior to the isolation process. Isolation efficiency and quality were analyzed by qPCR and qRT-PCR on the LightCycler® 480 Instrument II, respectively. Each isolation was performed in quadruplicates followed by a duplicated analysis on the LightCycler® 480 Instrument II. Therefore each value is calculated as the mean of 8 CP-values.

Sensitivity and linearity

In order to demonstrate the sensitivity of the High Pure Viral RNA Kit, 200 µL Citrate Plasma was spiked with decreasing amounts of HCV viral particles 1×10^2 to 1×10^7. Isolation was performed according to the Instructions for Use of the respective kit followed by quantitative analysis of HCV on the LightCycler® 480 Instrument II (see Fig. 2).

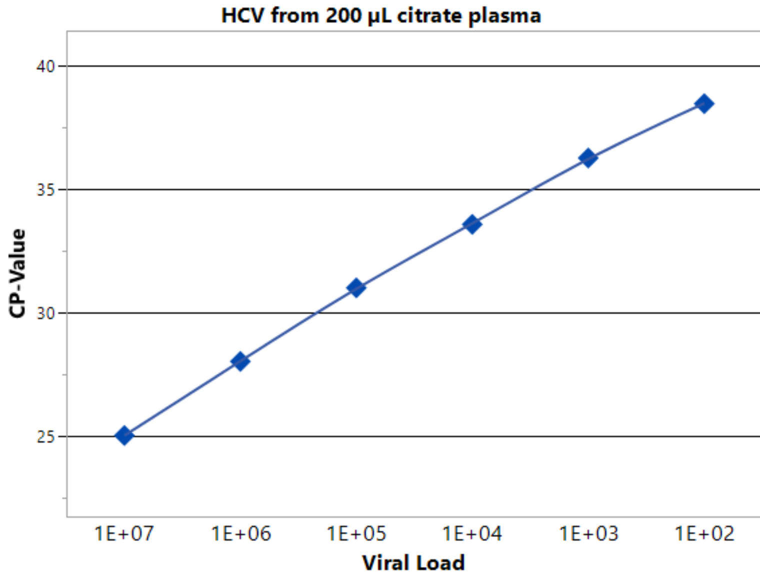

Fig. 2: Crossing Points of a series dilution of HCV particles in human Citrate Plasma after isolation with the High Pure Viral RNA Kit and subsequent analysis on the LightCycler® 480 Instrument II.

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.
	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 to avoid contamination.
		After reconstituting lyophilizate, aliquot and store at −15 to −25°C.
	Ethanol not added to Wash Buffer and Inhibitor 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 label Wash Buffer and Inhibitor Removal Buffer bottles to indicate whether ethanol has been added.
	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.	▪ Remove High Pure Filter Tube from tube containing eluted sample and spin sample for 1 minute 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.

5. Additional Information on this Product

5.1. Test Principle

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). 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. Fifty microliter eluate is sufficient for 8 to 14 RT-PCR reactions.
Included in the kit is a special Inhibitor Removal Buffer that results in improved sensitivity and reproducibility of RT-PCR assays performed with nucleic acid templates isolated with this kit. The use of the Inhibitor Removal Buffer allows even the application of heparinized sample material containing 100 U/mL heparin.

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 remove RT-PCR inhibitory contaminants.
– 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

For lot-specific certificates of analysis, see Section **Contact and Support**.

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.	
①②③ etc.	Stages in a process that usually occur in the order listed.
❶❷❸ 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.
Change of the constituent of Binding buffer in the Content Chapter.
Removed information related to the REACH Annex XIV.
Adapted the Result Chapter to show data derived when the new Binding Buffer is used.

6.3. Trademarks

LIGHTCYCLER is a trademark of Roche.
All other product names and trademarks are the property of their respective owners.

6.4. License Disclaimer

For additional documentation such as certificates and safety data sheets, please visit:
documentation.roche.com.

6.5. Regulatory Disclaimer

For general laboratory use.

6.6. Safety Data Sheet

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

6.7. 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.

Visit **documentation.roche.com**, to download or request copies of the following Materials:
- Instructions for Use
- Safety Data Sheets
- Certificates of Analysis
- Information Material

To call, write, fax, or email us, visit **lifescience.roche.com** and select your home country to display country-specific contact information.

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