For general laboratory use.

High Pure Viral Nucleic Acid Large Volume Kit

Version: 11
Content Version: July 2024

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

Cat. No. 05 114 403 001 1 kit
40 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 Nucleic Acid Large Volume Kit, Binding Buffer	Contains 6 M guanidine-HCl, 10 mM Tris-HCl, 10 mM urea, 20% polidocanol (w/v).	6 bottles, 25 mL each
2	–	High Pure Viral Nucleic Acid Large Volume Kit, Poly (A)	▪ For binding of RNA. ▪ Lyophilized	1 vial, 2 mg Poly (A) carrier RNA
3	pink	High Pure Viral Nucleic Acid Large Volume Kit, Proteinase K	▪ For the digestion of proteins. ▪ Lyophilized	2 vials, 100 mg each
4a	black	High Pure Viral Nucleic Acid Large Volume Kit, Inhibitor Removal Buffer	Contains 5 M guanidine-HCl, 20 mM Tris-HCl, pH 6.6 (+25°C); final concentration after addition of ethanol. *See Section* **Working Solution** *for information on preparing the solution.*	1 bottle, 33 mL
4	blue	High Pure Viral Nucleic Acid Large Volume Kit, Wash Buffer	Contains 20 mM NaCl, 2 mM Tris-HCl, pH 7.5 (+25°C); final concentrations after addition of ethanol. *See Section* **Working Solution** *for information on preparing the solution.*	1 bottle, 10 mL
5	colorless	High Pure Viral Nucleic Acid Large Volume Kit, Elution Buffer	Water, PCR Grade	1 bottle, 30 mL
6	–	High Pure Viral Nucleic Acid Large Volume Kit, High Pure Extender Assembly	For use of up to 2.5 mL sample volume.	8 bags, 5 pieces each in a single zip pack
7	–	High Pure Viral Nucleic Acid Large Volume Kit, Collection Tubes	For viral nucleic acid isolation.	2 bags, 50 polypropylene tubes, 2 mL each

⚠ ***All solutions are clear, except Bottle 1 Binding Buffer which can be clear to slightly turbid, and colorless to a slightly yellowish viscous solution. The buffers should not be used if precipitates are present. Warm the solutions at +15 to +25°C or in a +37°C water bath until the precipitates are dissolved.***

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 expiration date printed on the label.

Vial / bottle	Cap	Label	Storage
1	green	Binding Buffer	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.***
2	–	Poly (A)	
3	pink	Proteinase K	
4a	black	Inhibitor Removal Buffer	
4	blue	Wash Buffer	
5	colorless	Elution Buffer	
6	–	High Pure Extender Assembly	Store at +15 to +25°C.
7	–	Collection Tubes	

1.3. Additional Equipment and Reagent required

Standard laboratory equipment and reagents

- Absolute ethanol
- Standard tabletop centrifuge with swinging-bucket rotor capable of 5,000 × *g* centrifugal force for 50 mL polypropylene tubes
- Standard tabletop microcentrifuge capable of 13,000 × *g* centrifugal force
- Sterile microcentrifuge tubes, 1.5 mL

1.4. Application

The High Pure Viral Nucleic Acid Large Volume Kit is designed to isolate viral nucleic acids from up to 2.5 mL of mammalian serum, plasma, or whole blood.

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

1.5. Preparation Time

Assay Time

Total time	Approximately 25 minutes.
Hands-on time	10 minutes

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Isolation of viral nucleic acids from up to 2.5 mL:
- Serum
- Plasma
- Whole blood

⚠ ***Centrifuge samples containing precipitates before purification.***

Control Reactions

ⓘ *It is the user's own responsibility to apply 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.
- Use sterile disposable polypropylene tubes and tips to avoid RNase contamination. Always wear gloves during the assay.
- 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.
- Protect the plastic disposables from direct sunlight. Do not store the High Pure Extender Assembly near a window.
- 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...
Proteinase K (Vial 3)	Pipette 5.5 mL of Elution Buffer (Bottle 5) into the glass vial containing lyophilized Proteinase K. ▪ Add the rubber stopper and invert the vial until all the lyophilizate, including any lyophilizate stuck to the rubber stopper, is completely dissolved. ▪ Prepare 130 µL aliquots, labeling each aliquot with the date of reconstitution.	▪ Store aliquots at −15 to −25°C. ▪ Stable for 12 months.	Protocol, Step 1: Cell lysis
Poly (A) (Vial 2)	Dissolve Poly (A) carrier RNA (Vial 2) in 0.65 mL Elution Buffer (Bottle 5). ▪ Prepare 15 µL aliquots.	▪ Store aliquots at −15 to −25°C. ▪ Stable for 12 months.	For the preparation of the working solution.
	Working solution: Thaw one vial of 15 µL Poly (A) Carrier RNA and mix thoroughly with 0.5 mL to 2.5 mL Binding Buffer (Bottle 1), see the table in the **Isolation Protocol**.	⚠ ***Always prepare freshly before use; do not store.***	Protocol, Step 1
Inhibitor Removal Buffer (Bottle 4a)	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 6: To remove PCR Inhibitors.
Wash Buffer (Bottle 4)	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 the expiry date printed on kit label.	Protocol, Steps 8 and 9: Removal of residual impurities.

2.2. Protocols

Use of the High Pure Extender Assembly

The High Pure Extender Assembly is delivered in single zip bags. Five High Pure Extender Assemblies are additionally packed in labeled zip bags.

- Each High Pure Extender is assembled in a 50 mL polypropylene tube.
- The High Pure Extender Assembly is designed for use with tabletop centrifuges and swinging-bucket rotors with 4,000 × *g* force applicable.

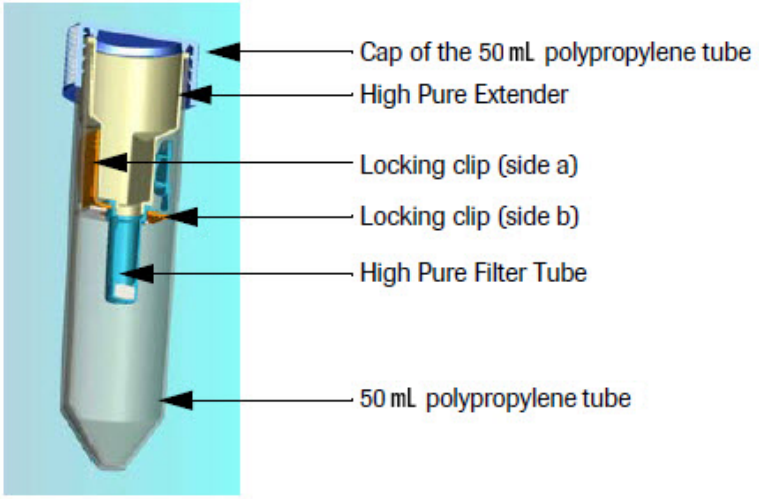

Fig. 1: High Pure Extender Assembly

1. Remove the High Pure Extender Assembly from the zip bags prior to use.
   – In order to load the sample onto the Assembly, unscrew the cap of the 50 mL polypropylene tube.
   – After sample loading, close the High Pure Extender Assembly with the 50 mL polypropylene tube cap.

2. After the first centrifugation step, the sample has passed through the High Pure Extender Assembly and is collected at the bottom of the 50 mL polypropylene tube.
   – Nucleic acids are bound to the silica fleece at the bottom of the High Pure filter tube.

3. For further processing, remove the High Pure Extender Assembly from the 50 mL polypropylene tube.

4. Discard the tube containing the flow through.

5. Remove the High Pure Filter Tube from the High Pure Extender Assembly (see Figures 2 to 6).

Disassembly of the High Pure Extender Assembly

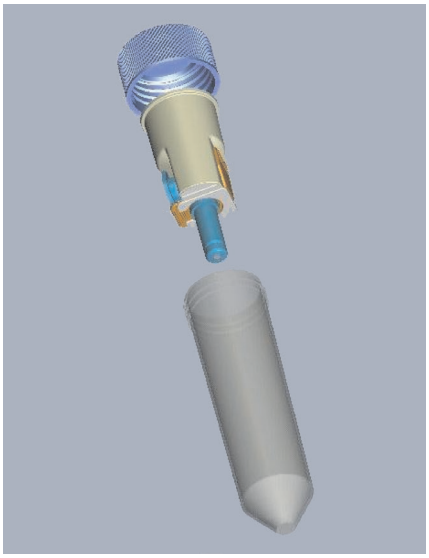

Fig. 2: Unscrew the 50 mL polypropylene tube and remove the High Pure Extender Assembly from the 50 mL polypropylene tube.

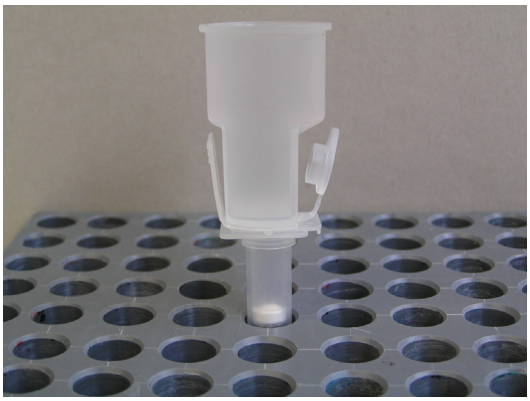

Fig. 3: Place the High Pure Extender into a new Collection Tube, which is placed securely in a tube rack on the bench.

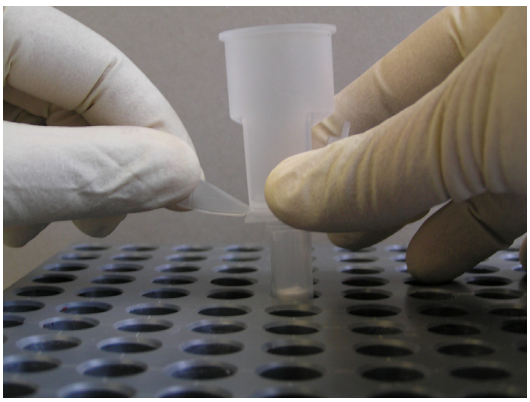

Fig. 4: Secure the High Pure Extender Assembly with one hand while grasping the locking clip (side a) of the High Pure Filter Tube cap on the opposite side with the other hand. Remove the first part of the locking clip (side a) by screwing the clip in either direction.

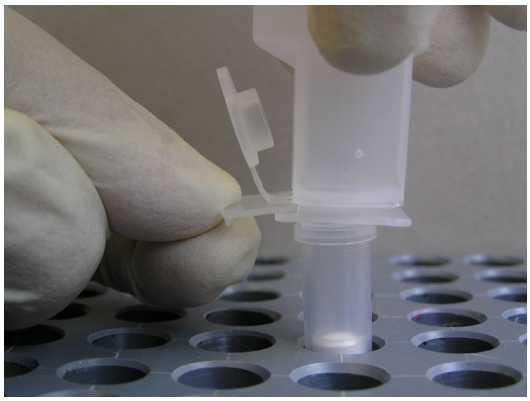

Fig. 5: Rotate the High Pure Extender Assembly. Remove the second part of the locking clip (side b) by pulling the locking clip away from the Extender Assembly.

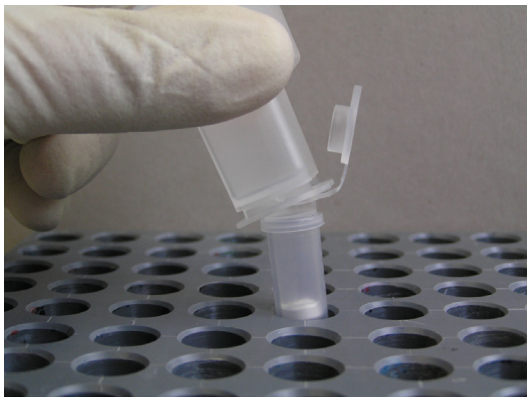

Fig. 6: Remove the High Pure Extender from the High Pure Filter Tube by tilting the High Pure Extender away from the High Pure Filter Tube toward the side without the cap.

⚠ ***We do not recommend the use of fixed-angle rotors in combination with the High Pure Extender Assembly. However, if you decide to use a centrifuge with fixed-angle rotors, do not centrifuge the High Pure Extender Assembly above 3,000 × g. The use of fixed-angle rotors results in incomplete flow of the liquid through the Assembly. The remaining sample solution therefore stays within the High Pure spin column Filter Tube. Remove this remaining liquid by an additional spin of the High Pure Filter Tube in a benchtop centrifuge before the first washing step. For this additional spin, remove the High Pure Spin Column from the High Pure Extender Assembly, according to Figures 2 to 6.***

Experimental overview

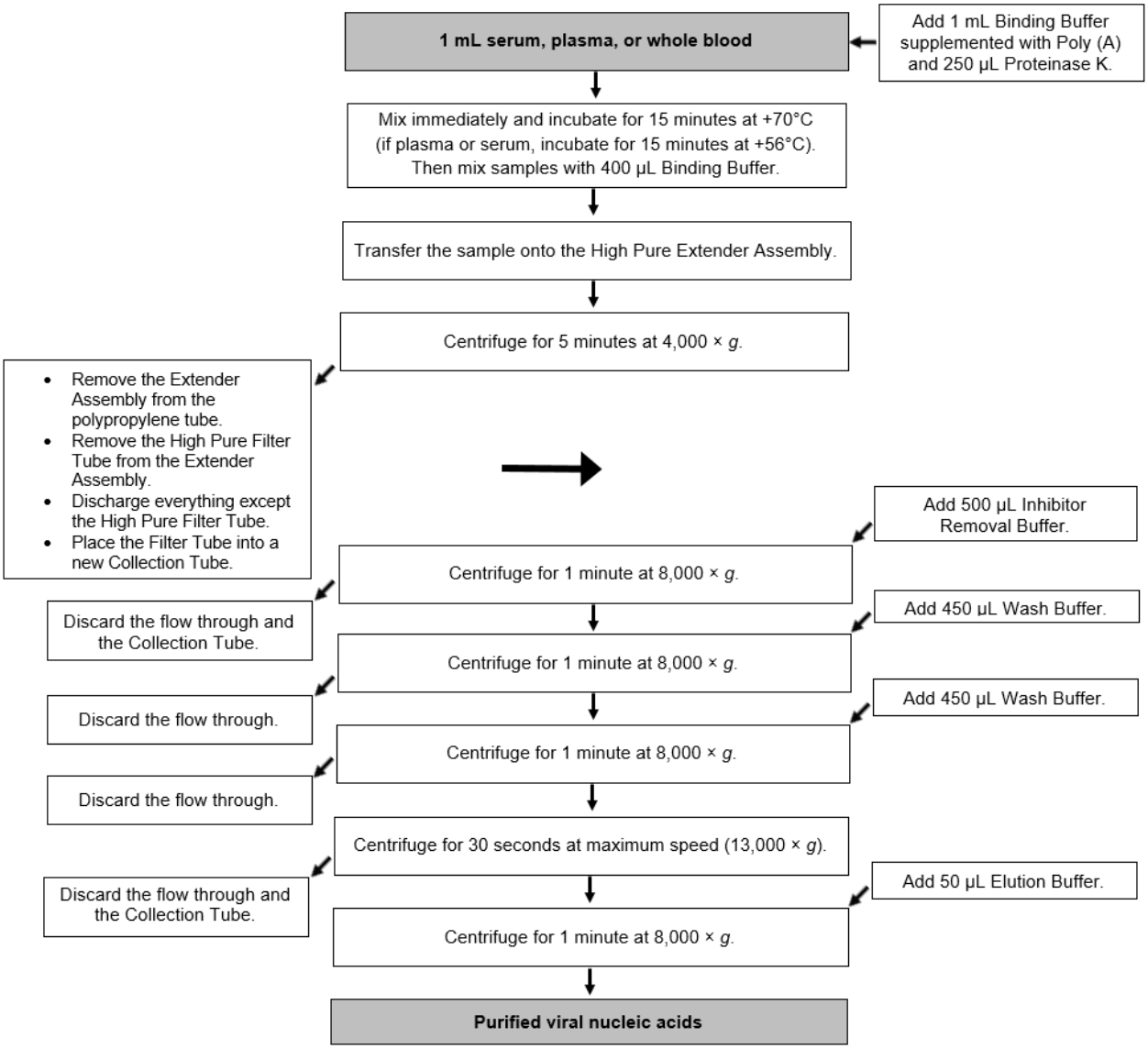

Fig. 7: Isolation of viral nucleic acids from a large volume of mammalian whole blood, serum, or plasma.

Isolation of nucleic acids from 1 mL serum, plasma, or whole blood

See Section **Working Solution** *for information on preparing solutions.*
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:
   – 1 mL serum, plasma, or whole blood.
   – 1 mL freshly prepared Working solution (carrier RNA-supplemented Binding Buffer).
   – 250 µL Proteinase K solution.
   – Mix immediately.
   – Incubate for 15 minutes at +70°C.

   If working with plasma or serum, incubate for 15 minutes at +56°C.

2. Add 400 µL Binding Buffer and mix.

3. To transfer the sample to a High Pure Extender Assembly, pipette the entire sample into the upper reservoir of the Assembly.

4. Insert the entire High Pure Filter Tube Assembly into a standard tabletop centrifuge with a swinging-bucket rotor.
   – Centrifuge for 5 minutes at 4,000 × *g*.

5. After centrifugation:
   – Remove the Filter Tube from the High Pure Extender Assembly; discard the flow through liquid and the 50 mL tube.
   – Insert the Filter Tube into a new Collection Tube.

6. After re-inserting the Filter Tube:
   – Add 500 µL Inhibitor Removal Buffer to the upper reservoir of the Filter Tube.
   – Centrifuge for 1 minute at 8,000 × *g*.

7. After centrifugation:
   – Remove the Filter Tube from the Collection Tube.
   – Insert the Filter Tube into a new Collection Tube; discard the Collection Tube including the flow through liquid.

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

9. After the first wash and centrifugation:
   – Remove the Filter Tube from the Collection Tube; discard the flow through liquid.
   – Insert the Filter Tube into a new Collection Tube.
   – Add 450 µL Wash Buffer to the upper reservoir of the Filter Tube.
   – Centrifuge for 1 minute at 8,000 × *g* and discard the flow through.
   – Leave the Filter Tube-Collection Tube assembly in the centrifuge and spin for 30 seconds at maximum speed (approximately 13,000 × *g*) to remove any residual Wash Buffer.

   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.

⓫ To elute the viral nucleic acids:
– Add 50 µL Elution Buffer to the upper reservoir of the Filter Tube.
– Incubate for 1 minute at +15 to +25°C.
– Centrifuge the tube assembly for 1 minute at 8,000 × *g*.

⓬ The microcentrifuge tube now contains the eluted viral nucleic acids.
– Use the eluted nucleic acids directly in PCR (10 to 20 µL DNA eluate) or RT-PCR (3.5 µL viral RNA). Alternatively, 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.

⚠ ***The High Pure Extender Assembly is designed for sample volumes up to 2.5 mL. The sample buffer compositions for different sample volumes are listed in the table below.***

Sample volume	0.5 mL	1 mL	2.5 mL
Binding Buffer	0.5 mL	1 mL	2.5 mL
Poly (A)	15 µL	15 µL	15 µL
Proteinase K	125 µL	250 µL	250 µL
Binding Buffer (Protocol, Step 2)	0.2 mL	0.4 mL	1 mL

3. Results

Sample materials and conditions

Validation of the High Pure Viral Nucleic Acid Large Volume Kit is accomplished with DNA Virus HBV and RNA Virus HCV samples.
Negative 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 Nucleic Acid Large Volume Kit, 1 mL Citrate Plasma was spiked with decreasing amounts of HBV viral particles 1 × 10^1 to 1 × 10^6 or with decreased 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 HBV and HCV on the LightCycler® 480 Instrument II.

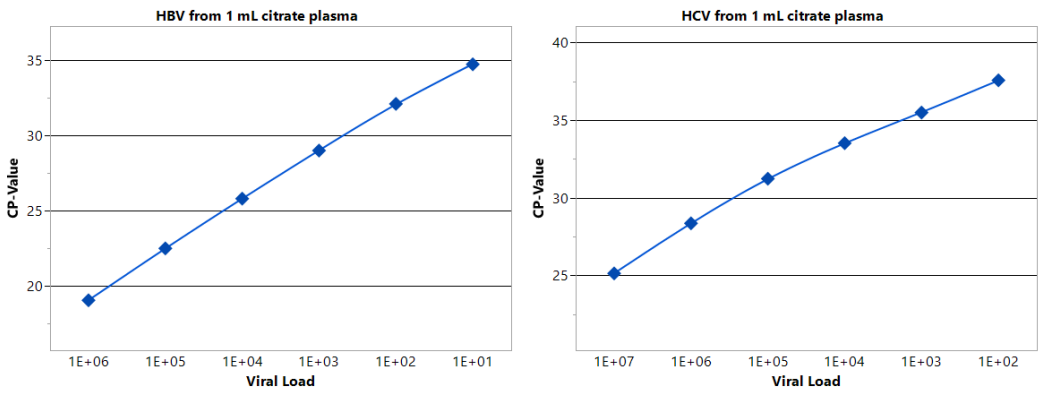

Fig. 8: Crossing Points of a series dilution of HBV and HCV particles in human Citrate Plasma after isolation with the High Pure Viral Nucleic Acid Large Volume Kit and subsequent analysis on the LightCycler® 480 Instrument II.

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 lyophilizates, 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.
	Low temperature during Proteinase K digestion.	Check temperature during Proteinase K digestion. Heating blocks for 15 mL polypropylene tubes may deliver lower temperatures inside the tube.
		Raise temperature of the heating block until +70°C is reached inside the 15 mL tube.
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 co-elute 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.
	Incomplete Proteinase K digestion.	Be sure to dissolve the lyophilized Proteinase K completely. *See Section,* **Working Solution** *for additional information.*

5. Additional Information on this Product

5.1. Test Principle

How this product works

As a pre-requisite for the analysis of viral nucleic acids by 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. 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 surface of the glass fibers, thereby promoting adsorption to the glass fiber fleece. 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.

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

The typing error in Section **Working Solution** for the reconstitution of Proteinase K has been corrected to 5.5 mL.
Editorial changes.

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

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