For general laboratory use.

# High Pure Viral Nucleic Acid Large Volume Kit

Content Version: October 2020

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 | Binding Buffer | Contains 6 M guanidine-HCl, 10 mM Tris-HCl, 10 mM urea, 20% -Triton X-100 w/v, pH 4.4 +25°C | ▪ 6 × 25 ml |
| 2 | | PolyA | Lyophilizate poly(A) carrier RNA for binding of RNA | ▪ 2 mg |
| 3 | pink | Proteinase K | Lyophilizate for the digestion of proteins | ▪ 2 × 100 mg |
| 4a | black | Inhibitor Removal Buffer | Contains 5 M guanidine-HCl, 20 mM Tris-HCl, pH 6.6 [(+25°C) final concentration after addition of ethanol] | ▪ 33 ml, add 20 ml absolute ethanol |
| 4 | blue | Wash Buffer | Contains 20 mM NaCl, 2 mM Tris-HCl, pH 7.5 +25°C final concentrations after addition of ethanol | ▪ 10 ml, add 40 ml absolute ethanol each case |
| 5 | colorless | Elution Buffer | Water, PCR Grade | ▪ 30 ml |
| 6 | | High Pure Extender Assembly | | ▪ 8 bags, 5 pieces each in a single zip pack |
| 7 | | Collection Tubes | | ▪ 2 bags with 50 polypropylene tubes 2 ml |

⚠ ***All solutions are clear except Vial 1, Binding Buffer, which is clear to slightly turbid, 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)

⚠ ***The High Pure Viral Nucleic Acid Large Volume 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.***

⚠ ***Improper storage at +2 to +8°C (refrigerator) or −15 to −25°C (freezer) will adversely impact nucleic acid purification due to formation of precipitates in the solutions.***

### Storage Conditions (Working Solution)

| Solution | Storage |
|---|---|
| PolyA carrier RNA solution | -15 to -25°C |
| Proteinase K | -15 to -25°C |

## 1.3. Additional Equipment and Reagent required

- Absolute ethanol
- Standard tabletop centrifuge with swing-bucket rotor capable of 5,000 × *g* centrifugal force for 50 ml polypropylene tubes
- Standard tabletop microcentrifuge capable of 13,000 × *g* centrifugal force
- Microcentrifuge tubes, 1.5 ml, sterile

## 1.4. Application

The High Pure Viral Nucleic Acid Large Volume Kit is designed for the purification of viral nucleic acids from up to 2.5 ml of 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.

## 1.5. Preparation Time

### Assay Time

| | |
|---|---|
| **Total time** | Approx. 25 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 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 control concept.***

### General Considerations

#### Handling Requirements

⚠ ***Binding Buffer and Inhibitor Removal Buffer contain guanidinium hydrochloride which is an irritant. Always wear gloves and follow standard safety precautions to minimize contact when handling.***
⚠ ***Do not allow Binding and Inhibitor Removal Buffer to touch your skin, eyes, or mucous membranes. If contact does occur, wash the affected area immediately with large amounts of water. 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.***
⚠ ***Do not use any modified ethanol.***
⚠ ***Do not pool reagents from different lots.***
⚠ ***Use sterile disposable polypropylene tubes and tips to avoid RNase contamination. Always wear gloves during the assay.***
⚠ ***Do not allow the Binding Buffer and Inhibitor Removal Buffer to mix with sodium hypchlorite found in commercial bleach solutions. This mixture can produce a highly toxic gas.***

### Safety Information

#### Laboratory Procedures

- Handle all samples as potentially infectious, using safe laboratory procedures. 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.
- 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 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.
- Protect the plastic disposables from direct sun light. Do not store the High Pure Extender Assembly near a window.
- 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.
- 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 set-up. 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.

## For customers in the European Economic Area

Contains SVHC: octyl/nonylphenol ethoxylates. For use in research and under controlled conditions only – acc. to Art. 56.3 and 3.23 REACH Regulation.

# Working Solution

In addition to the ready-to-use solutions supplied with this kit, prepare the following working solutions:

| Content | Reconstitution / Preparation | Storage / Stability | For use in... |
|---|---|---|---|
| Proteinase K<br>Vial 3; pink cap | Dissolve Proteinase K in 5.5 ml Elution Buffer and mix thouroghly.<br>Prepare 130 µl aliquots. | ▪ Store aliquots at −15 to −25°C<br>▪ Stable for 12 months. | Protocol step 1:<br>Cell lysis |
| PolyA carrier RNA<br>Vial 2 | Dissolve poly(A) carrier RNA vial 2 in 0.65 ml Elution Buffer Vial 5.<br>Prepare 15 µl aliquots. | ▪ Store aliquots at −15 to −25°C<br>▪ Stable for 12 months. | For the preparation of the working solution |
| | Working solution:<br>Thaw one vial with 15 µl PolyA Carrier RNA and mix thoroughly with 0.5 ml to 2.5 ml Binding Buffer Vial 1 according to table listed in the isolation protocol. | ⚠ ***Always prepare freshly before use! Do not store!*** | Protocol step 1 |
| Inhibitor Removal Buffer<br>Vial 4a; black cap | Add 20 ml absolute ethanol to Inhibitor Removal Buffer and mix well.<br>⚠ ***Label and date bottle accordingly after adding ethanol.*** | ▪ Store at +15 to +25°C.<br>▪ Stable until expiration date printed on kit label. | Protocol step 6:<br>Removal of PCR Inhibitors |
| Wash Buffer<br>Vial 4; blue cap | Add 40 ml absolute ethanol to each Wash Buffer and mix well.<br>⚠ ***Label and date bottle accordingly after adding ethanol.*** | ▪ Store at +15 to +25°C.<br>▪ Stable until expiration date printed on kit label. | Protocol step 8 and 9:<br>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 table-top centrifuges and swing-bucket rotors with 4,000 × g force applicable.

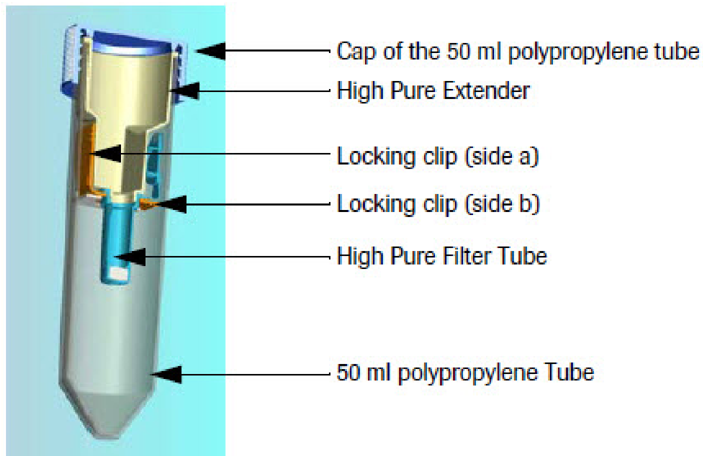



**Fig. 1:** High Pure Extender Assembly

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.
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. For further processing remove the High Pure Extender Assembly from the 50 ml polypropylene tube. Discard the tube containing the flow-through. Remove the High Pure Filter Tube from the High Pure Extender Assembly (see Figures 2 to 6).

### Disassembly of the High Pure Extender Assembly

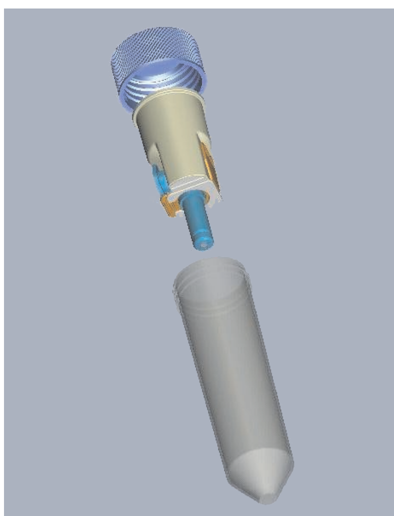


**Fig. 2:** Unscrew the 50 ml polypropylene tube and remove the High Pure Extender Assembly from the 50 ml polypropylene tube.

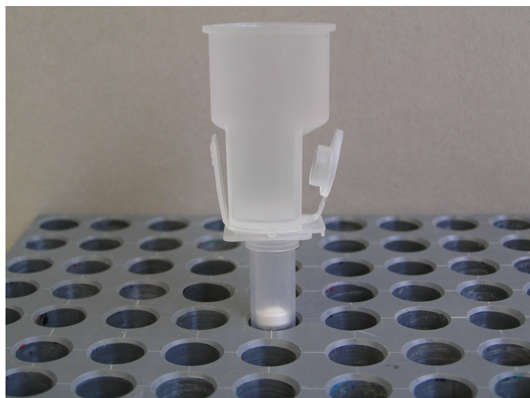


**Fig. 3:** Place the High Pure Extender in a new collection tube, which is placed securely in a tube rack on the bench.

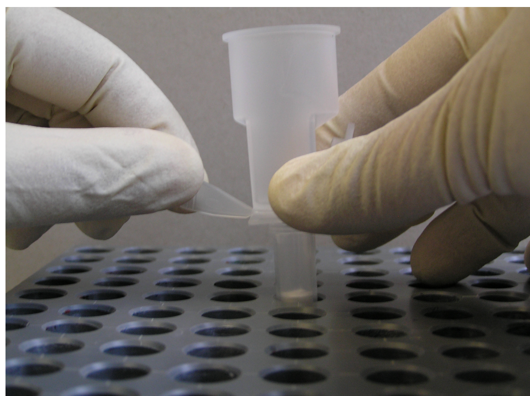


**Fig. 4:** Secure the High Pure Extender Assembly with one hand while grasping the locking clip (side a) of the High Pure Filter Tube cap on the opposite side with the other hand. Remove the first part of the locking clip (side a) by screwing the clip in either direction.

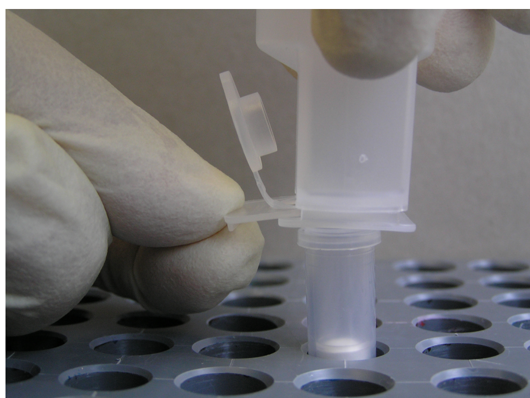


**Fig. 5:** Rotate the High Pure Extender Assembly. Remove the second part of the locking clip (side b) by pulling the locking clip away from the Extender Assembly.

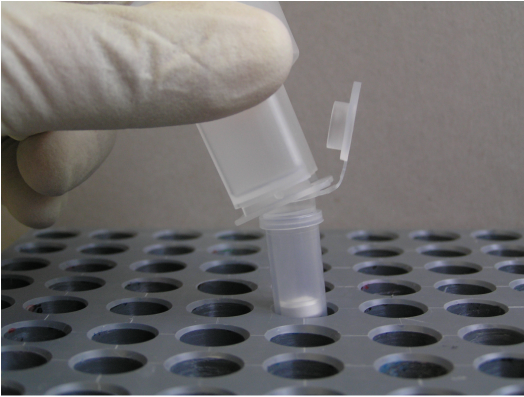
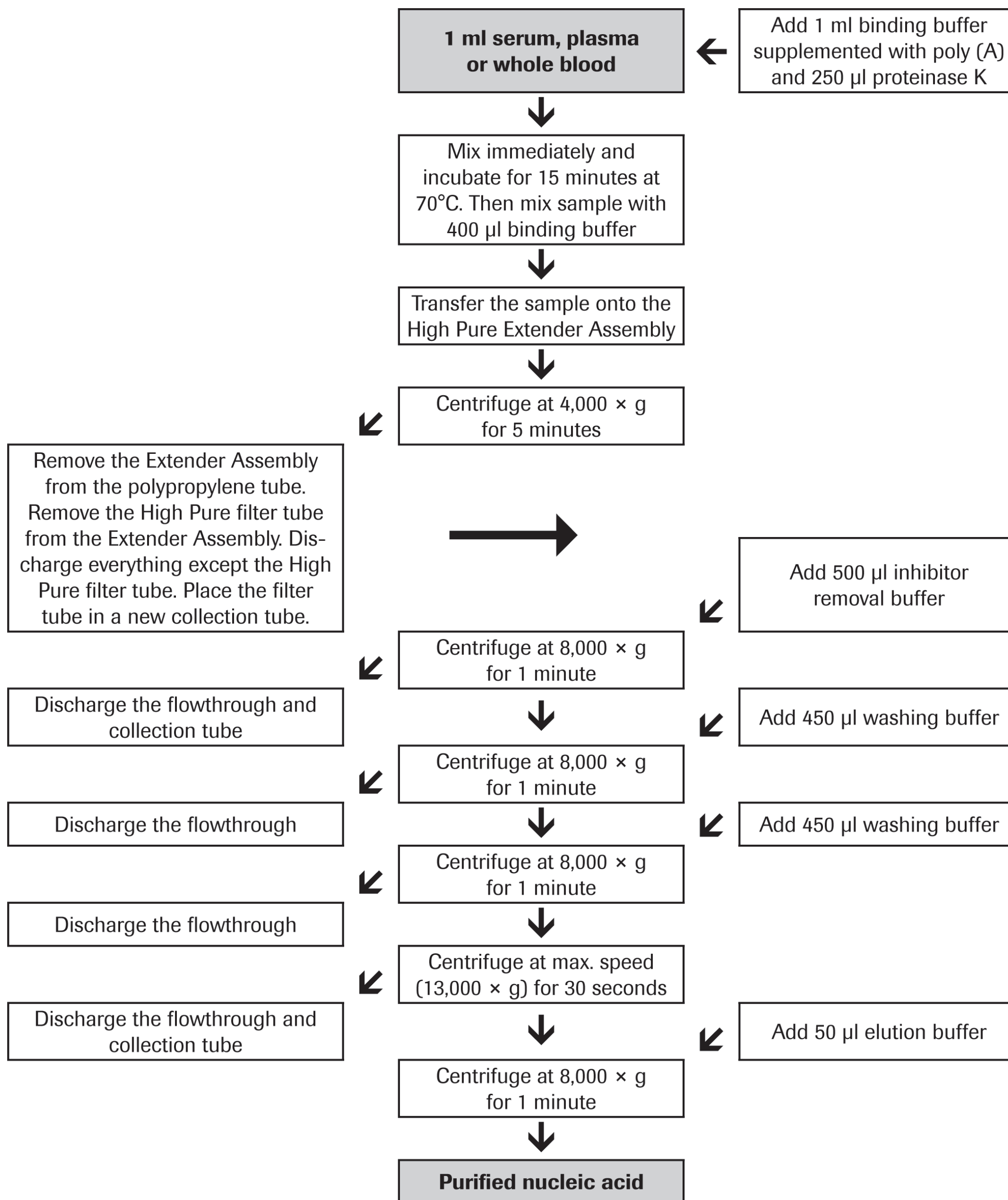


**Fig. 6:** Remove the High Pure Extender from the High Pure Filter Tube by tilting the High Pure Extender away from the High Pure Filter Tube toward the side without the cap.

⚠ ***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 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 bench-top centrifuge before the first washing step. For this additional spin remove the High Pure Spin Column from the High Pure Extender Assembly (according to Figure 2 to 6).***

## Experimental Overview



## Isolation Protocol

### Procedure for Preparing Nucleic Acids from 1 ml Samples of Serum, Plasma or Whole Blood

1. Add the following to a nuclease-free 15 ml tube:
   - 1 ml serum, plasma or whole blood
   - 1 ml working solution, freshly prepared, [carrier RNA-supplemented Binding Buffer]
   - 250 µl Proteinase K solution
   - Mix immediately
   - Incubate for 15 minutes at +70°C

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2. Add 400 µl Binding Buffer and mix.

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

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

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5. After centrifugation:
   - Remove the Filter Tube from the High Pure Extender Assembly, discard the flow-through liquid and the 50 ml tube.
   - Combine the Filter Tube with a new Collection Tube.

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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 for 1 minute at 8,000 × *g*.

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7. After centrifugation:
   - Remove the Filter Tube from the Collection Tube.
   - Combine the Filter Tube with a new Collection Tube.
   - Discard the Collection Tube including the flow-through liquid.

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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.

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9. After the first wash and centrifugation:
   - Remove the Filter Tube from the Collection Tube, discard the flow-through liquid.
   - Combine the Filter Tube with a new Collection Tube.
   - Add 450 ml 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.

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

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

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

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ℹ *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*

⚠ ***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 |

⚠ ***For isolation of nucleic acids from whole blood use pre-warmed Elution Buffer +70°C.***

# 3. Results

## Sample Materials and Conditions

Validation of the High Pure Viral Nucleic Acid Kit is accomplished with DNA Virus EBV and RNA Virus HAV samples. Negative human samples Serum, Citrate Plasma, and EDTA whole blood 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® 2.0 Instrument, respectively. Each isolation was performed in triplicate followed by a duplicated analysis on the LightCycler® 2.0 Instrument. Therefore each value is calculated as the mean of 6 CP-values.

## Sensitivity and Linearity

In order to demonstrate the sensitivity of the High Pure Viral Nucleic Acid Large Volume Kit, 1 ml Citrated Plasma was spiked with decreasing amounts of HAV viral particles $1 \times 10^5$ to $1 \times 10^2$. Isolation was performed according to the Instructions for Use of the respective kit followed by quantitative analysis of HAV on the LightCycler® 2.0 Instrument

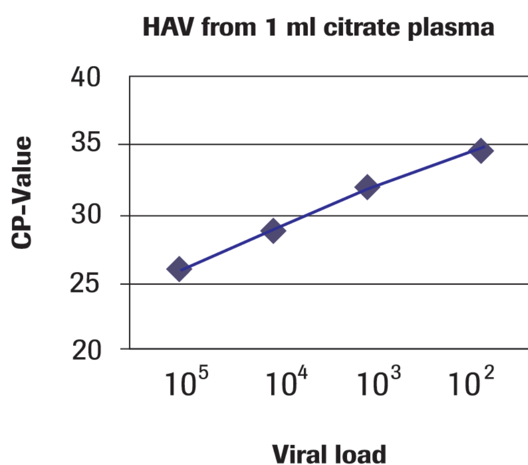


**Fig. 7:** Crossing Points of a series dilution of HAV particles in human citrate plasma after isolation with the High Pure Viral Nucleic Acid Large Volume Kit and subsequent analysis on the LightCycler® 2.0 Instrument.
As shown in Figure 7 we observe a high sensitivity and good linearity for HAV detection in Citrate Plasma down to 100 copies per 1 ml of sample volume.

## Comparison with Other Products

For the comparison with other products, 1 ml of negative human serum was spiked with a dilution series $1 \times 10^6$ to $1 \times 10^4$ of HAV or EBV virus particles, followed by isolation according to the Instructions for Use of the respective kits. Isolation efficiency and quality were analyzed by qPCR and qRT-PCR on the LightCycler® 2.0 Instrument utilizing a quantitative analysis of HAV or LightCycler® EBV Quantification Kit (Roche Molecular Diagnostics, Cat. No. 04 846 818 001), respectively.

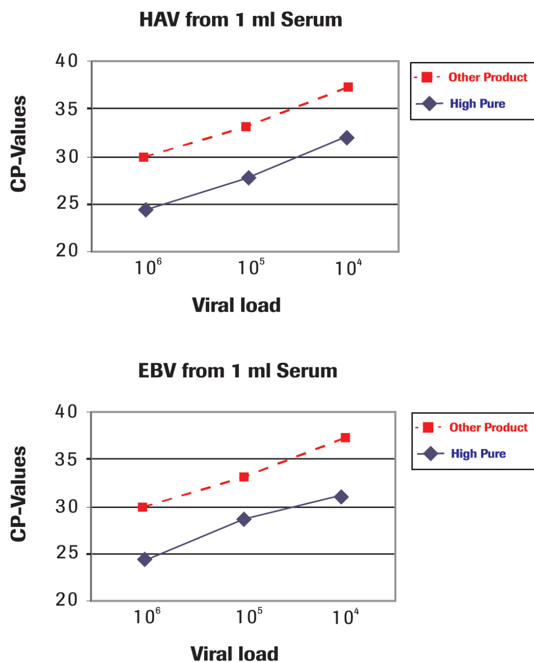


**Fig. 8:** Crossing Points of a series dilution of HAV and EBV particles in human serum after isolation with the High Pure Viral nucleic Acid Large Volume Kit and subsequent analysis on the LightCycler® 2.0 Instrument.

As shown in Figure 8, a higher sensitivity and excellent linearity for HAV and EBV detection in 1 ml human serum is observed compared with another product.

# 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. |
| | Low temperature at Proteinase K digest. | Check temperature during Proteinase K digestion. Heating blocks for 15 ml polypropylene tubes might 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.<br>▪ 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:<br>⚠ ***Reconstituted Proteinase K is stable for 12 months when stored properly.***<br>▪ Pipet 5.5 ml of Elution Buffer into the glass vial containing lyophilized Proteinase K.<br>▪ Replace stopper and invert the vial until all the lyophilizate including any stuck to the rubber stopper is dissolved.<br>▪ Aliquot the reconstituted enzyme, mark each aliquot with the date of reconstitution, and store at -15 to -25° C. |

# 5. Additional Information on this Product

## 5.1. Test Principle

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

A dilution series of the Epstein-Barr Virus (EBV) sequence containing plasmids is prepared in 1 ml human plasma, applied to the filter tubes, washed, and eluted according to the kit protocol. 5 µl of the eluate is analyzed by an EBV-specific qPCR using the LightCycler® System. Down to $10^4$ copies of EBV plasmid DNA are detected.

# 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

New information added related to the REACH Annex XIV

## 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 patent license limitations for individual products please refer to:
**http://technical-support.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 **lifescience.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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