ViroReal® Kit HDV

Manual

For research use only

REF **DHUV00953**

Σ **50 reactions**

ingenetix GmbH
Arsenalstraße 11
1030 Vienna, Austria
T +43(0)1 36 1980 198
F +43(0)1 36 1980 199
office@ingenetix.com
www.ingenetix.com

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Explanation of symbols

LOT	Batch code		Use by
REF	Catalogue number		Manufactured by
Σ	Contains sufficient for <n> tests		Store at
	Corrosion, GHS05		Exclamation mark, GHS07

1. Intended use

ViroReal® Kit HDV is a test, based on one-step reverse transcription real-time PCR (RT-PCR), for the detection of human hepatitis D virus.

2. Product description

ViroReal® Kit HDV detects RNA of the genus hepatitis D virus.
This test allows the rapid and sensitive detection of RNA of HDV from samples purified from blood (e.g. with the QIAamp Viral RNA Mini Kit, Qiagen).

A probe-specific amplification-curve in the FAM channel indicates the amplification of HDV specific RNA. An internal RNA positive control (RNA IPC) is detected in Cy5 channel and is used as RNA extraction as well as RT-PCR inhibition control. The target for the RNA IPC is extracted with the sample.

This test has been validated with the Applied Biosystems® 7500 Fast Real-time PCR System (Thermo Fisher Scientific) and tested with a LightCycler® 480 Instrument II (Roche) and Mx3005P® QPCR System (Agilent), but is also compatible with other real-time PCR instruments which detect and differentiate fluorescence in FAM and Cy5 channel.

The test is based on one-step reverse transcription real-time PCR (RT-PCR). A specific RNA sequence of the pathogen genome is transcribed into cDNA and amplified in a one-step PCR. The generated PCR-product is detected by an oligonucleotide-probe labelled with a fluorescent dye. This technology allows a sequence-specific detection of PCR amplificates.

BactoReal®, MycoReal, ParoReal and ViroReal® Kits are optimized to run under the same thermal cycling conditions. RNA and DNA material can be analysed in one run.

3. Pathogen information

Hepatitis D Virus is considered a subviral satellite which depends on Hepatitis B Virus for propagation. Thus it is exclusively affecting patients with already existing Hepatitis B infection. The virus has a worldwide distribution (especially genotype 1, referred to as HDV-I); certain genotypes are endemic in distinct areas, such as the Far East (genotypes IIA and IIB, referred to as HDV-2 and HDV-4, respectively), South America (genotype III, referred to as HDV-3) and Western and Central Africa (genotypes 5, 6, and 7, referred to as HDV-5, HDV-6, and HDV-7, respectively). HDV is causative agent of fulminant hepatitis and may aggravate chronic hepatitis B infection resulting in cirrhosis and liver failure. It is transmitted through body fluids.

Literature:
Rizzetto M. 2015. Hepatitis D Virus: Introduction and Epidemiology. Cold Spring Harb Perspect Med. 5(7).

4. Contents of the kit, stability and storage

Labelling	Content	Amount	Storage
HDV Assay Mix (green cap)	Primer and probe (FAM) for HDV detection	1 x 50 µl	-15 °C to -25 °C
RNA IPC-3 Assay Mix (yellow cap)	Primer and probe (Cy5) for RNA IPC detection	1 x 50 µl	-15 °C to -25 °C
RNA IPC Target (orange cap)	Target for RNA IPC (internal RNA positive control system)	1 x 100 µl	-15 °C to -25 °C
HDV Positive Control (red cap)	DNA positive control	1 x 25 µl	-15 °C to -25 °C
RNA Reaction Mix (white cap)	Amplification mix for one-step RT real-time PCR	1 x 250 µl	-15 °C to -25 °C
Nuclease-free water (blue cap)	Nuclease-free water	1 x 1000 µl	-15 °C to -25 °C

The components of ViroReal® Kit HDV are stable until the expiry date stated on the label.

5. Additionally required materials and devices

- Reagents and devices for RNA-extraction
- Nuclease-free water for dilution of RNA IPC Target
- Disposable powder-free gloves
- Pipettes (adjustable)
- Sterile pipette tips with filters
- Vortex mixer
- Desktop centrifuge with rotor for 2 ml reaction tubes
- Real-time PCR instrument which is able to detect and differentiate fluorescence in FAM and Cy5 channel
- Optical 96 well reaction plates or optical reaction tubes

6. Precautions and safety information

- The use of this kit is limited to persons instructed in the procedures of real-time PCR.
- Clean benches and devices periodically.
- Use sterile filter pipette tips.
- Specimens should be handled as if infectious in accordance with safe laboratory procedures. Wear protective disposable powder-free gloves when handling kit reagents and specimens.
- Use separated working areas for specimen preparation, reagent preparation and amplification. Supplies and equipment must be dedicated to each of these separate working areas and ensure workflow in the laboratory from pre- to post-PCR.
- Be careful when handling samples and positive control to avoid cross contamination. Change gloves after handling of samples or positive control. Store positive or potentially positive material separately from reagents
- Prevent contamination of work equipment and reagents with DNA/RNA, nuclease or amplification products by good laboratory practice.
- Quality of RNA has a profound impact on the test performance. Ensure that the used RNA extraction system is compatible with reverse transcription real-time PCR technology.
- Always include a negative control per PCR-run (nuclease-free water instead of sample).
- For a valid interpretation of results, a negative control should be included during RNA-extraction (e.g. extraction of water instead of sample material), in order to exclude false-positive results due to contamination with virus RNA during extraction.
- Please note the expiry date of the kit.
- Do not interchange or mix reagents from kits with different lot numbers.
- Repeated thawing and freezing of kit components should be avoided. Protect kit components from light. **Caution:** RNA IPC Target is stored in RNA stabilizer which contains Guanidinium thiocyanate/Triton X-100 (see MSDS, www.ingenetix.com).
- Use established laboratory practices according to your local safety regulations for discarding specimens, reagents and waste.

7. Limitations

- Optimal performance of this test requires appropriate specimen collection, transport and storage, as well as an appropriate RNA extraction procedure.
- A negative test result does not exclude the possibility of HDV infection, because test results may be affected by improper specimen collection, technical error, specimen mix-up or viral quantities below the assay sensitivity. The presence of PCR inhibitors may lead to invalid results.
- For this kit highly specific primers and probes have been selected. However, false-negative or less sensitive results might be obtained due to sequence heterogeneity within the target region of not yet described clinical subtypes.
- Results should be interpreted in consideration of clinical and laboratory findings.

8. Preparation of samples

Extract samples with a RNA extraction system compatible with reverse transcription real-time PCR technology. An extraction negative control should be included during RNA-extraction (e.g. extraction of water instead of sample material).
The **RNA IPC Target** has to be added during extraction. The RNA IPC is used as a control of RNA extraction, identifies possible PCR inhibition and confirms the integrity of kit reagents.
Caution: The RNA IPC Target must not be added directly to the sample material but has to be pipetted to the lysis buffer.
→ For an elution volume of 50-100 µl: Per sample, spike 1 µl RNA IPC Target into lysis buffer.
→ For an elution volume of >100 µl or when using an automated extraction system: Per sample, spike 2 µl RNA IPC Target into lysis buffer.

Use RNA immediately after extraction (always store on ice) and store at -80°C as soon as possible.

9. Preparation of real-time PCR

- Include one negative control (water), one positive control and one extraction negative control per PCR run.
- It is recommended to analyse samples in duplicates, which increases the probability of pathogen detection and facilitates interpretation of results.
- Thaw RNA samples on ice.
- Thaw RNA Reaction Mix on ice. Invert the RNA Reaction Mix 2 to 3 times to ensure homogeneity of solution. Avoid warming to room temperature. Thaw all other kit components thoroughly at room temperature. When thawed, mix components, centrifuge briefly and keep on ice.
- Prepare master mix on ice.
- **Positive Control**
  → Use 1 µl of HDV Positive Control + 9 µl nuclease-free water.
  Pipette positive control at last.

9.1. Pipetting scheme

		Per sample
Preparation of Master Mix (mix well)	Nuclease-free Water*	3.0 µl
	RNA Reaction Mix	5.0 µl
	HDV Assay Mix	1.0 µl
	RNA IPC-3 Assay Mix	1.0 µl
	Total volume Master Mix	**10.0 µl**
Preparation of PCR	Master Mix	10.0 µl
	RNA-Sample*	10.0 µl
	Total volume	**20.0 µl**

*1-10 µl of the sample can be used. When using < 10 µl sample, the volume of water has to be adjusted accordingly.

→ **If RNA IPC Target was not added during extraction:** Freshly dilute the RNA IPC Target 1:500 with nuclease-free water and add 1 µl per sample directly to the master mix.
Caution: The use of more than 1 µl diluted (1:500) RNA IPC Target per reaction causes inhibition of the RT-PCR reaction.

9.2. Programming of temperature profile

Please find further information on programming the real-time PCR instrument in the respective operator's manual. Take into consideration that some PCR-platforms have to be calibrated with the corresponding dye before performing a multiplex-PCR.

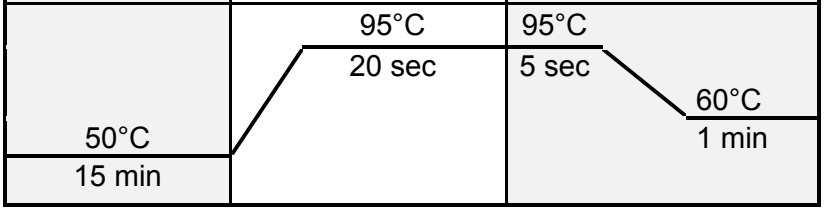
Select detection channel: FAM-TAMRA, 530 nm (for HDV)
Cy5-NONE, 667 nm (for RNA IPC-3)

Select reference dye (passive reference): ROX

Sample Volume: 20 µl

Temperature Profile:

Program 1	**Program 2**	**Program 3**
Cycles: 1 Analysis: None	Cycles: 1 Analysis: None	Cycles: 45 Analysis: Quantification Acquisition at 60°
50°C 15 min	95°C 20 sec	95°C 5 sec 60°C 1 min



For ABI PRISM® 7500:
Ramp speed: "Standard"

For LightCycler® 480 instrument:
Detection format: 2 Color Hydrolysis Probe

Note: These parameters are valid for all ingenetix ViroReal®, BactoReal®, MycoReal and ParoReal kits.

10. Interpretation of PCR-data

For analysis of PCR results select fluorescence display options 530 nm (FAM channel) for the HDV target and 667 nm (Cy5 channel) for the RNA IPC target. Samples with positive Ct or Cp-values are considered positive. Please additionally check the amplification-curves manually. Samples should be inspected both in logarithmic and linear scale view and compared with the negative control.

	FAM channel HDV target	**Cy5 channel RNA IPC target**	**Interpretation**
Negative control	Negative	Negative / Positive[1]	Valid
Positive control	Positive	Negative / Positive[1]	Valid
Extraction negative control	Negative	Positive	Valid
Sample	Positive	Positive / Negative[2]	Positive
Sample	Negative	Positive	Negative[3]
Sample	Negative	Negative	Invalid

[1]Only positive if the RNA IPC Target was added at a 1:500 dilution directly to the master mix
[2]High virus load in the sample can lead to reduced or absent signal of RNA IPC
[3]The positive signal of the RNA IPC excludes a possible PCR inhibition. However, IPC Ct-values should show comparable results. A shift of Ct-values can indicate a partial inhibition of PCR.

In case of invalid data, analysis has to be repeated with the remaining or newly extracted RNA sample (see 11. Troubleshooting).

11. Troubleshooting

11.1. No signal in FAM channel and Cy5 channel with controls and sample:

- Incorrect programming of the temperature profile or detection channels of the real-time PCR instrument.
  → Compare temperature profile and programming of detection channels with the protocol.
- Incorrect configuration of PCR reaction.
  → Check your work steps (see pipetting scheme) and repeat PCR, if necessary.
- RNA might be degraded.

11.2. Valid results for controls, but no signal in FAM channel and Cy5 channel with sample:

- Incorrect programming of detection channels with the sample.
  → Compare programming of detection channels with protocol.
- RNA might be degraded.
- If the RNA IPC Target was added during extraction:
  - PCR reaction has been inhibited.
  - RNA extraction has failed.
  - The undiluted RNA IPC Target has not been added to lysis buffer of sample.
  - The extracted sample has not been added to PCR reaction.

  → No interpretation can be made. Make sure you use a recommended method for RNA isolation and stick closely to the manufacturer's instructions. Check your work steps.

11.3. Signal in FAM channel in negative control:

- A contamination occurred during preparation of PCR.
  → Repeat PCR with new reagents in replicates.
  → Strictly pipette positive control at last.
  → Make sure that work space and instruments are cleaned at regular intervals.

11.4. Signal in FAM channel in extraction negative control:

- A contamination occurred during extraction.
  → Repeat extraction and PCR using new reagents.
  → Make sure that work space and instruments are cleaned at regular intervals.

12. Kit performance

Performance of ViroReal® Kit HDV with an Applied Biosystems® 7500 Fast Real-time PCR System (Thermo Fisher Scientific) is shown in Figure 1.

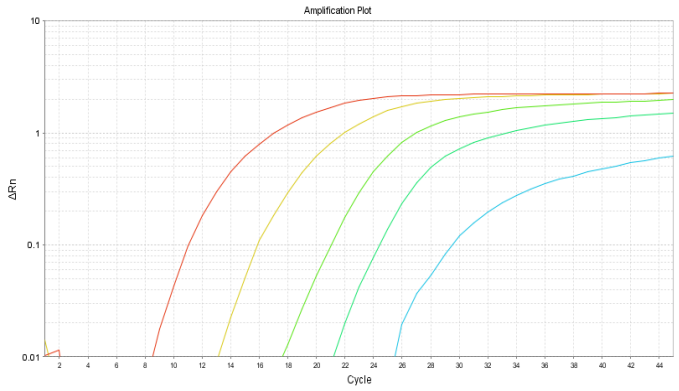
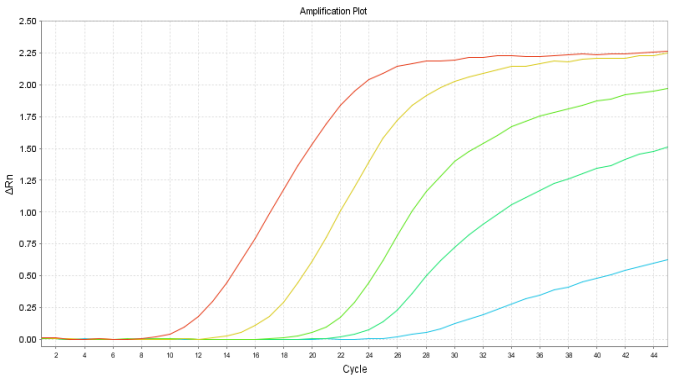
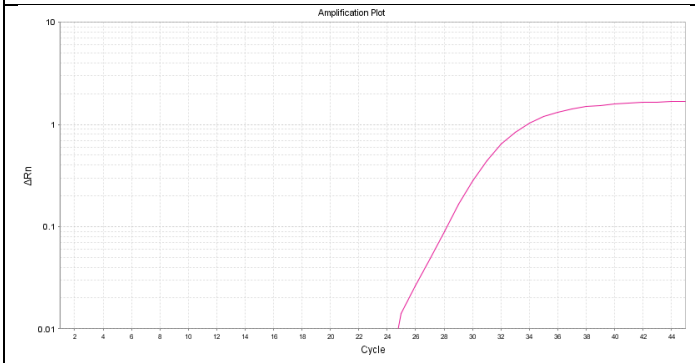
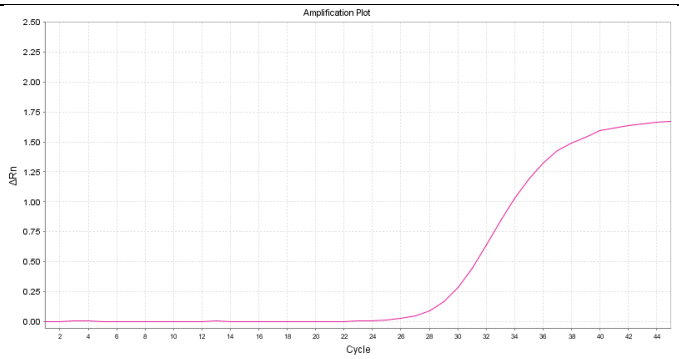
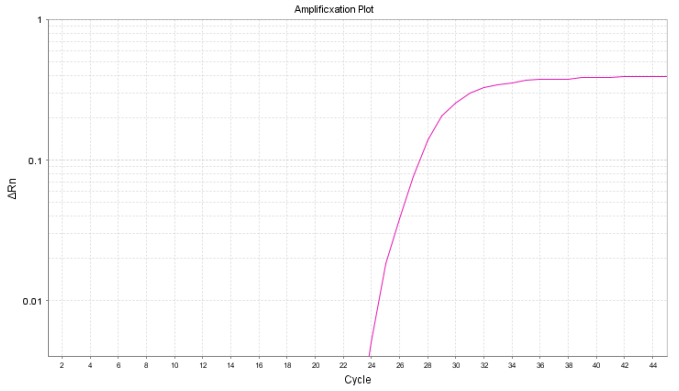
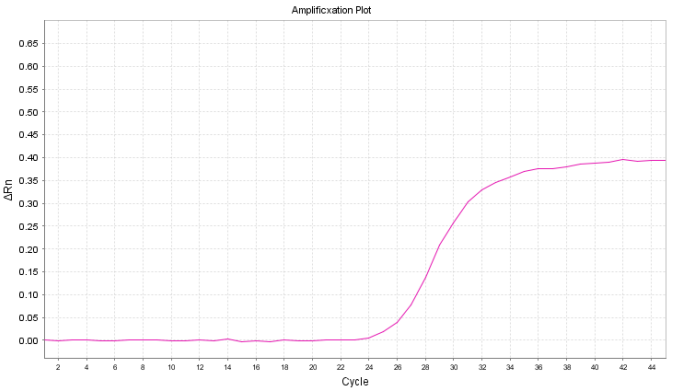
Logarithmic view	Linear view
	
ABI Prism® 7500: FAM channel, 530 nm 1:10 serial dilution of HDV target	
	
ABI Prism® 7500: FAM channel, 530 nm HDV positive control	
	
ABI Prism® 7500: Cy5 channel, 667 nm Internal RNA positive control	

Figure 1 Performance of ViroReal® Kit HDV