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Instructions for Use

RealStar®
HDV RT-PCR Kit 1.0

05/2018 EN

RealStar®

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HDV RT-PCR Kit 1.0

For research use only!

(RUO)

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Content

1. Application

The RealStar® HDV RT-PCR Kit 1.0 is a reagent system, based on real-time PCR technology, for the detection and quantification of hepatitis D virus specific RNA.

For research use only (RUO)! Not for use in diagnostic procedures.

2. Kit Components

Lid Color	Component	Number of Vials	Volume [µl/Vial]
Blue	Master A	8	60
Purple	Master B	8	240
Green	Internal Control	1	1000
Red	QS1-4*	1	550
White	Water (PCR grade)	1	500

* The RealStar® HDV RT-PCR Kit 1.0 contains Quantification Standards (QS) at four different concentrations (see Chapter 4. Product Description)

3. Storage

- The RealStar® HDV RT-PCR Kit 1.0 is shipped on dry ice. The components of the kit should arrive frozen. If one or more components are not frozen upon receipt, or if tubes have been compromised during shipment, contact altona Diagnostics GmbH for assistance.
- All components should be stored between -25°C and -15°C upon arrival.
- Repeated thawing and freezing of Master reagents (more than twice) should be avoided, as this might affect the performance of the assay. The reagents should be frozen in aliquots, if they are to be used intermittently.
- Storage between +2°C and +8°C should not exceed a period of two hours.
- Protect Master A and Master B from light.

4. Product Description

The RealStar® HDV RT-PCR Kit 1.0 is a reagent system, based on real-time PCR technology, for the detection and quantification of hepatitis D virus specific RNA.

The assay includes a heterologous amplification system (Internal Control) to identify possible RT-PCR inhibition and to confirm the integrity of the reagents of the kit.

Real-time RT-PCR technology utilizes reverse-transcriptase (RT) reaction to convert RNA into complementary DNA (cDNA), polymerase chain reaction (PCR) for the amplification of specific target sequences and target specific probes for the detection of the amplified DNA. The probes are labelled with fluorescent reporter and quencher dyes.

Probes specific for HDV RNA are labelled with the fluorophore FAM™. The probe specific for the Internal Control (IC) is labelled with the fluorophore JOE™.

Using probes linked to distinguishable dyes enables the parallel detection of HDV specific RNA and the Internal Control in corresponding detector channels of the real-time PCR instrument.

The test consists of three processes in a single tube assay:

- Reverse transcription of target and Internal Control RNA to cDNA
- PCR amplification of target and Internal Control cDNA
- Simultaneous detection of PCR amplicons by fluorescent dye labelled probes

The RealStar® HDV RT-PCR Kit 1.0 consists of:

- Two Master reagents (Master A and Master B)
- Internal Control (IC)
- Four Quantification Standards (QS1 - QS4)
- PCR grade water

Master A and Master B contain all components (PCR buffer, reverse transcriptase, DNA polymerase, magnesium salt, primers and probes) to allow reverse transcription, PCR mediated amplification and detection of HDV specific RNA and Internal Control in one reaction setup.

The Quantification Standards contain standardized concentrations of HDV specific RNA. These Quantification Standards were calibrated against the 1st WHO International Standard for Hepatitis D Virus for Nucleic Acid Amplification Techniques (NAT) (PEI code 7657/12). The Quantification Standards can be used individually as positive controls, or together to generate a **standard curve**, which can be used to determine the concentration of HDV specific RNA in a sample.

The Quantification Standards have the following concentrations:

Quantification Standard	Concentration [IU/µl]
QS1	1000
QS2	100
QS3	10
QS4	1

4.1 Real-Time PCR Instruments

The RealStar® HDV RT-PCR Kit 1.0 can be used with the following real-time PCR instruments:

- VERSANT® kPCR Molecular System AD (Siemens Healthcare Diagnostics)
- ABI Prism® 7500 SDS (Applied Biosystems)
- Rotor-Gene® 6000 (Corbett Research)
- Rotor-Gene® Q5/6 plex Platform (QIAGEN)
- CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad)
- LightCycler® 480 Instrument II (Roche)

NOTE	
	***Please ensure that all instruments used have been installed, calibrated, checked and maintained according to the manufacturer´s instructions and recommendations.***

5. Procedure

5.1 Sample Preparation

Extracted RNA is the starting material for the RealStar® HDV RT-PCR Kit 1.0.

The quality of the extracted RNA has a profound impact on the performance of the entire test system. It has to be ensured that the system used for nucleic acid extraction is compatible with real-time PCR technology. The following kits and systems are suitable for nucleic acid extraction:

- QIAamp® Viral RNA Mini Kit (QIAGEN)
- QIAsymphony® (QIAGEN)
- NucliSENS® easyMag® (bioMérieux)
- MagNA Pure 96 System (Roche)
- m2000sp (Abbott)
- Maxwell® 16 IVD Instrument (Promega)
- VERSANT® kPCR Molecular System SP (Siemens Healthcare)

Alternative nucleic acid extraction systems and kits might also be appropriate.

If using a spin column based sample preparation procedure including washing buffers containing ethanol, it is highly recommended to perform an additional centrifugation step for 10 min at approximately 17000 x g (~ 13000 rpm), using a new collection tube, prior to the elution of the nucleic acid.

CAUTION	
	***If your sample preparation system is using washing buffers containing ethanol, make sure to eliminate any traces of ethanol prior to elution of the nucleic acid. Ethanol is a strong inhibitor of real-time PCR.***
	***The use of carrier RNA is crucial for extraction efficiency and stability of the extracted nucleic acid.***

For additional information and technical support regarding pre-treatment and sample preparation please contact our Technical Support (see chapter 8. Technical Assistance).

5.2 Master Mix Setup

All reagents and samples should be thawed completely, mixed (by pipetting or gentle vortexing) and centrifuged briefly before use.

The RealStar® HDV RT-PCR Kit 1.0 contains a heterologous Internal Control (IC), which can either be used as a RT-PCR inhibition control or as a control of the sample preparation procedure (nucleic acid extraction) and as a RT-PCR inhibition control.

► If the IC is used as a RT-PCR inhibition control, but not as a control for the sample preparation procedure, set up the Master Mix according to the following pipetting scheme:

Number of Reactions (rxns)	1	12
Master A	5 µl	60 µl
Master B	20 µl	240 µl
Internal Control	2.5 µl	30 µl
Volume Master Mix	**27.5 µl**	**330 µl**

- If the IC is used as a control for the sample preparation procedure and as a RT-PCR inhibition control, add the IC during the nucleic acid extraction procedure.
- No matter which method/system is used for nucleic acid extraction, the IC **must not** be added directly to the sample. The IC should always be added to the sample/lysis buffer mixture. The volume of the IC which has to be added, always and only depends on the elution volume. It represents 10% of the elution volume. For instance, if the nucleic acid is going to be eluted in 60 µl of elution buffer or water, 6 µl of IC per sample must be added into the sample/lysis buffer mixture.
- If the IC was added during the sample preparation procedure, set up the Master Mix according to the following pipetting scheme:

Number of Reactions (rxns)	1	12
Master A	5 µl	60 µl
Master B	20 µl	240 µl
Volume Master Mix	**25 µl**	**300 µl**

CAUTION

If the IC (Internal Control) was added during the sample preparation procedure, at least the negative control must include the IC.

CAUTION

No matter which method/system is used for nucleic acid extraction, never add the IC directly to the sample.

5.3 Reaction Setup

- ► Pipette 25 µl of the Master Mix into each required well of an appropriate optical 96-well reaction plate or an appropriate optical reaction tube.
- ► Pre-heating/Initial Denaturation

As HDV has a partially double-stranded RNA, it is recommended to perform a "pre-heating" of the eluates before use in Real-time PCR.

1. Close the lever lid tubes with the eluates additionally with parafilm or use screw-cap tubes
2. Place the tubes with the eluates on a heating block at 95°C for 3 minutes
3. Centrifuge the tubes gently to remove drops from inside the lid
4. Place the tubes immediately on ice
5. Use a cooling block for the preparation of the Master Mix and addition of the eluates

- ► Add 25 µl of the sample (eluate from the nucleic acid extraction) or 25 µl of the controls (Quantification Standard, Positive or Negative Control).

Reaction Setup	
Master Mix	25 µl
Sample or Control	25 µl
Total Volume	**50 µl**

- ► Make sure that at least one Positive (QS) and one Negative Control is used per run.

- ► For quantification purposes all Quantification Standards (QS1 to QS4) should be used.
- ► Thoroughly mix the samples and controls with the Master Mix by pipetting up and down.
- ► Close the 96-well reaction plate with appropriate lids or optical adhesive film and the reaction tubes with appropriate lids.
- ► Centrifuge the 96-well reaction plate in a centrifuge with a microtiter plate rotor for 30 seconds at approximately 1000 x g (~ 3000 rpm).

NOTE

Pre-heating of eluates is recommended before use in real-time PCR.

6. Programming the Real-Time PCR Instrument

For basic information regarding the setup and programming of the different real-time PCR instruments, please refer to the user manual of the respective instrument.

For detailed programming instructions regarding the use of the RealStar® HDV RT-PCR Kit 1.0 on specific real-time PCR instruments please contact our Technical Support (see chapter 8. Technical Assistance).

6.1 Settings

- ► Define the following settings:

Settings	
Reaction Volume	50 µl
Ramp Rate	Default
Passive Reference	ROX™

6.2 Fluorescence Detectors (Dyes)

► Define the fluorescence detectors (dyes):

Target	Detector Name	Reporter	Quencher
HDV specific RNA	HDV	FAM™	(None)
Internal Control (IC)	IC	JOE™	(None)

6.3 Temperature Profile and Dye Acquisition

► Define the temperature profile and dye acquisition:

	Stage	Cycle Repeats	Acquisition	Temperature [°C]	Time [min:sec]
Reverse Transcription	Hold	1	-	55	20:00
Denaturation	Hold	1	-	95	02:00
Amplification	Cycling	45	-	95	00:15
			yes	55	00:45
			-	72	00:15

7. Data Analysis

For basic information regarding data analysis on specific real-time PCR instruments, please refer to the user manual of the respective instrument.

For detailed instructions regarding the analysis of the data generated with the RealStar® HDV RT-PCR Kit 1.0 on different real-time PCR instruments please contact our Technical Support (see chapter 8. Technical Assistance).

7.1 Interpretation of Results

7.1.1 Qualitative Analysis

Detection Channel		Result Interpretation
FAM™	JOE™	
+	+*	HDV specific RNA detected.
-	+	No HDV specific RNA detected. Sample does not contain detectable amounts of HDV specific RNA.
-	-	RT-PCR inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample.

* Detection of the Internal Control in the JOE™ detection channel is not required for positive results in the FAM™ detection channel. A high HDV RNA load in the sample can lead to a reduced or absent Internal Control signal.

7.1.2 Quantitative Analysis

The RealStar® HDV RT-PCR Kit 1.0 includes four Quantification Standards (QS). In order to generate a **standard curve** for quantitative analysis, these have to be defined as **standards** with appropriate concentrations (see chapter 4. Product Description). Using **standards** of known concentrations a standard curve for quantitative analysis can be generated.

$$C_t = m \cdot \log (N_0) + b$$

C_t = Threshold Cycle

m = Slope

N_0 = Initial Concentration

b = Intercept

Derived from the standard curve positive samples of unknown concentrations can be quantified.

$$N_0 = 10^{(C_t - b)/m}$$

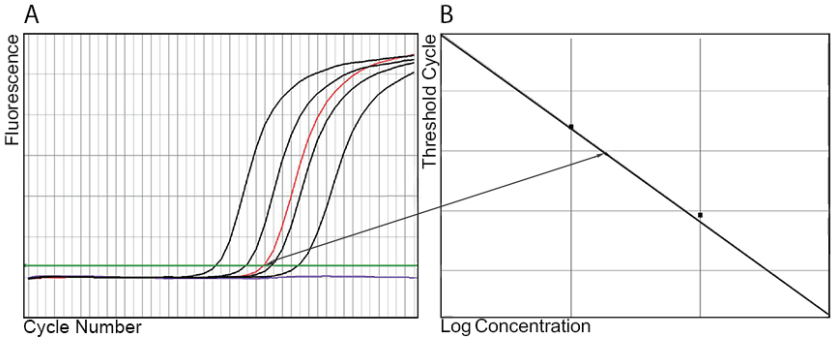

Figure 1: Quantification Standards (black), a positive (red) and a negative sample (blue) displayed in the Amplification Plot [**A**] and Standard Curve analysis [**B**]

NOTE

The concentration of the "Sample" is displayed in IU/µl and refers to the concentration in the eluate.

To determine the **viral load of the original sample**, the following formula has to be applied:

$$\text{Viral load (}\mathbf{Sample}\text{) [IU/ml]} = \frac{\text{Volume (}\mathbf{Eluate}\text{) [µl]} \cdot \text{Viral load (}\mathbf{Eluate}\text{) [IU/µl]}}{\text{Sample Input [ml]}}$$

8. Technical Assistance

For technical advice, please contact our Technical Support:

e-mail: **support@altona-diagnostics.com**
phone: **+49-(0)40-5480676-0**

9. Trademarks and Disclaimers

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10. Explanation of Symbols

Symbol	Explanation
RUO	For Research Use Only
LOT	Batch code
CAP	Cap color
REF	Product number
CONT	Content
NUM	Number
COMP	Component
	Consult instructions for use
	Contains sufficient for “n” tests/reactions (rxns)
	Temperature limit
	Use-by date
	Manufacturer
	Caution
	Note
	Version

Notes:

always a drop ahead.

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