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Instructions for Use

RealStar®
Hantavirus-HFRS RT-PCR Kit 1.0

08/2022 EN

RealStar®

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Hantavirus-HFRS RT-PCR Kit 1.0

For research use only!

(RUO)

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Content

1. Application

The RealStar® Hantavirus-HFRS RT-PCR Kit 1.0 is a reagent system, based on real-time PCR technology, for the qualitative detection and differentiation of RNA specific for hantavirus causing hemorrhagic fever with renal syndrome (HFRS). The assay is designed to detect and differentiate the following four hantavirus-HFRS species: Dobrava-Belgrade hantavirus (DOBV), Seoul hantavirus (SEOV), Hantaan hantavirus (HTNV) and Puumala hantavirus (PUUV).

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

2. Kit Components

The kit contains two different RT-PCR assays with 48 reactions each. It includes two different Positive Controls (PC): one for the Dobrava-Belgrade hantavirus (DOBV) and Seoul hantavirus (SEOV) specific amplification and detection system and another for the Hantaan hantavirus (HTNV) and Puumala hantavirus (PUUV) specific amplification and detection system.

Table 1: Kit components

Lid color	Component	Number of vials	Volume [µl/vial]
Blue	Master A DOBV & SEOV	4	60
Light blue	Master A HTNV & PUUV	4	60
Purple	Master B DOBV & SEOV	4	180
Light purple	Master B HTNV & PUUV	4	180
Green	Internal Control	1	1000
Red	PC DOBV & SEOV	1	250
Orange	PC HTNV & PUUV	1	250
White	Water (PCR grade)	1	500

3. Storage

- The RealStar® Hantavirus-HFRS 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 at -25 °C to -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 at +2 °C to +8 °C should not exceed a period of 2 hours.
- Protect Master A and Master B from light.

4. Product Description

The RealStar® Hantavirus-HFRS RT-PCR Kit 1.0 is a reagent system, based on real-time PCR technology, for the qualitative detection and differentiation of RNA specific for hantavirus causing hemorrhagic fever with renal syndrome (HFRS). The assay is designed to detect and differentiate the following four hantavirus-HFRS species: Dobrava-Belgrade hantavirus (DOBV), Seoul hantavirus (SEOV), Hantaan hantavirus (HTNV) and Puumala hantavirus (PUUV).

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.

The RealStar® Hantavirus-HFRS RT-PCR Kit 1.0 consists of two independent assays, one targeting Dobrava-Belgrade hantavirus (DOBV) and Seoul hantavirus (SEOV) specific RNA and another targeting Hantaan hantavirus (HTNV) and Puumala hantavirus (PUUV) specific RNA.

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 labeled with fluorescent reporter and quencher dyes.

Master DOBV & SEOV: The probe specific for DOBV RNA is labeled with the fluorophore Cy5 and the probe specific for SEOV RNA is labeled with the fluorophore FAM™.

Master HTNV & PUUV: The probe specific for HTNV RNA is labeled with the fluorophore FAM™ and the probe specific for PUUV RNA is labeled with the fluorophore Cy5.

The probe specific for the Internal Control (IC) is labeled with the fluorophore JOE™.

Using probes linked to distinguishable dyes enables the parallel detection of SEOV and DOBV respectively HTNV and PUUV specific RNA, as well as the detection of the IC in the 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 labeled probes

The RealStar® Hantavirus-HFRS RT-PCR Kit 1.0 consists of:

- Master A DOBV & SEOV
- Master A HTNV & PUUV
- Master B DOBV & SEOV
- Master B HTNV & PUUV
- Internal Control

- PC DOBV & SEOV
- PC HTNV & PUUV
- Water (PCR grade)

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

4.1 Real-Time PCR Instruments

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

- ABI Prism® 7500 SDS (Applied Biosystems)
- CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad)
- CFX96™ Real-Time PCR Detection System (Bio-Rad)
- LightCycler® 480 Instrument II (Roche)
- QuantStudio™ 5 Real-Time PCR System (Applied Biosystems)
- Rotor-Gene® Q5/6 plex Platform (QIAGEN)

NOTE	
i	***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® Hantavirus-HFRS RT-PCR Kit 1.0.

The quality of the extracted RNA has a profound impact on the performance of the entire test system. It is recommended to ensure 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 1 min at approximately 17,000 x g (~ 13,000 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.***

CAUTION

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® Hantavirus-HFRS 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 Mixes according to the following pipetting scheme:

Number of Reactions (rxns)	1	12
Master A	5 µl	60 µl
Master B	15 µl	180 µl
Internal Control	1 µl	12 µl
Volume Master Mix	**21 µl**	**252 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 Mixes according to the following pipetting scheme:

Number of Reactions (rxns)	1	12
Master A	5 µl	60 µl
Master B	15 µl	180 µl
Volume Master Mix	**20 µl**	**240 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 20 µl of the Master Mix DOBV & SEOV or the Master Mix HTNV & PUUV into each required well of an appropriate optical 96-well reaction plate or an appropriate optical reaction tube.
- ► Add 10 µl of the sample (eluate from the nucleic acid extraction) or 10 µl of the controls (Positive or Negative Control).

Reaction Setup	
Master Mix	20 µl
Sample or Control	10 µl
Total Volume	**30 µl**

- ► Make sure that each Positive Control and at least one Negative Control is used per run.
- ► 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 1,000 x g (~ 3,000 rpm).

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® Hantavirus-HFRS 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	30 µl
Ramp Rate	Default
Passive Reference	None

6.2 Fluorescence Detectors (Dyes)

► Define the fluorescence detectors (dyes):

Target	Master Mix	Detector Name	Reporter	Quencher
SEOV specific RNA	DOBV & SEOV	SEOV	FAM™	(None)
DOBV specific RNA		DOBV	Cy5	(None)
HTNV specific RNA	HTNV & PUUV	HTNV	FAM™	(None)
PUUV specific RNA		PUUV	Cy5	(None)
Internal Control (IC)	DOBV & SEOV and HTNV & PUUV	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:s]
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® Hantavirus-HFRS 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

Table 2: Qualitative analysis SEOV & DOBV

Detection Channel			Result Interpretation
FAM™ (SEOV)	Cy5 (DOBV)	JOE™ (IC)	
+	+	+*	SEOV and DOBV specific RNA detected.
+	-	+*	SEOV specific RNA detected.
-	+	+*	DOBV specific RNA detected.
-	-	+	Neither SEOV nor DOBV specific RNA detected. The sample does not contain detectable amounts of SEOV and/or DOBV 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 neither in the FAM™ nor in the Cy5 detection channel. A high SEOV and/or DOBV RNA load in the sample can lead to a reduced or absent Internal Control signal.

Table 3: Qualitative analysis HTNV & PUUV

Detection Channel			Result Interpretation
FAM™ (HTNV)	Cy5 (PUUV)	JOE™ (IC)	
+	+	+*	HTNV and PUUV specific RNA detected.
+	-	+*	HTNV specific RNA detected.
-	+	+*	PUUV specific RNA detected.
-	-	+	Neither HTNV nor PUUV specific RNA detected. The sample does not contain detectable amounts of HTNV and/or PUUV 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 neither in the FAM™ nor in the Cy5 detection channel. A high HTNV and/or PUUV RNA load in the sample can lead to a reduced or absent Internal Control signal.

8. Technical Assistance

For customer support, please contact our Technical Support:

e-mail: **support@altona-diagnostics.com**

phone: **+49-(0)40-5480676-0**

9. Trademarks and Disclaimers

RealStar® (altona Diagnostics); ABI Prism®, QuantStudio™ (Applied Biosystems); easyMAG®, NucliSENS® (bioMérieux); CFX96™ (Bio-Rad); FAM™, JOE™ (Life Technologies); Maxwell® (Promega); QIAamp®, QIAsymphony®, Rotor-Gene® (QIAGEN); LightCycler® (Roche), VERSANT® (Siemens Healthcare).

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

10. Explanation of Symbols

Symbol	Explanation
RUO	For Research Use Only
LOT	Batch code
CAP	Cap color
REF	Catalogue 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: Highlights operating instructions or procedures which, of not followed correctly, may result in personal injury or impact product performance. Contact altona Diagnostics Technical Support for assistance.

Symbol	Explanation
	Note: Information is given to the user that is useful but not essential to the task at hand.
	Version

always a drop ahead.

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