altona DIAGNOSTICS

Instructions for Use

RealStar®
Filovirus Type RT-PCR Kit 2.0

03/2021 EN

RealStar®

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Filovirus Type RT-PCR Kit 2.0

For research use only!

(RUO)

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Content

1. Application

The RealStar® Filovirus Type RT-PCR Kit 2.0 is a reagent system, based on real-time PCR technology, for the qualitative detection and differentiation of filovirus specific RNA. The assay is designed to detect *Ebola*- and *Marburgvirus* and differentiate the five *Ebolavirus* species: *Bundibugyo ebolavirus* (BDBV), *Reston ebolavirus* (RESTV), *Sudan ebolavirus* (SUDV), *Tai Forest ebolavirus* (TAFV) and *Zaire ebolavirus* (EBOV).

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

2. Kit Components

The kit contains 4 different RT-PCR assays with 24 reactions each. It contains five different Ebolavirus controls representing each species and one Positive Control for Marburgvirus.

Lid Color	Component	Number of Vials	Volume [µl/Vial]
Blue	Master A EBOV	2	60
Blue	Master A MARV	2	60
Blue	Master A RESTV/ TAFV	2	60
Blue	Master A SUDV/ BDBV	2	60
Purple	Master B EBOV	2	180
Purple	Master B MARV	2	180
Purple	Master B RESTV/ TAFV	2	180
Purple	Master B SUDV/ BDBV	2	180
Green	Internal Control	1	1000
Red	Positive Control EBOV	1	250

Lid Color	Component	Number of Vials	Volume [µl/Vial]
Red	Positive Control MARV	1	250
Red	Positive Control RESTV	1	250
Red	Positive Control TAFV	1	250
Red	Positive Control SUDV	1	250
Red	Positive Control BDBV	1	250
White	Water (PCR grade)	1	500

3. Storage

- The RealStar® Filovirus Type RT-PCR Kit 2.0 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® Filovirus Type RT-PCR Kit 2.0 is a reagent system, based on real-time PCR technology, for the qualitative detection and differentiation of filovirus specific RNA. The assay is designed to detect *Ebola-* and *Marburgvirus* and differentiate the five *Ebolavirus* species: *Bundibugyo ebolavirus* (BDBV), *Reston ebolavirus* (RESTV), *Sudan ebolavirus* (SUDV), *Tai Forest ebolavirus* (TAFV) and *Zaire ebolavirus* (EBOV).

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 labeled with fluorescent reporter and quencher dyes.

Using probes linked to distinguishable dyes enables the parallel detection and discrimination of the respective Ebola- or Marburgvirus specific RNA as well as the detection of Internal Control in the corresponding detector channels of the real-time PCR instrument.

The kit contains four different master PCR-reagents:

Master 1 detects EBOV which is labeled with the fluorophore FAM™.

Master 2 detects MARV which is labeled with the fluorophore FAM™.

Master 3 detects RESTV and TAFV, probes specific for RESTV are labeled with the fluorophore FAM™, whereas the probes specific for TAFV are labeled with a fluorophore showing similar characteristics to Cy5.

Master 4 detects SUDV and BDBV, probes specific for SUDV are labeled with the fluorophore FAM™, whereas the probes specific for BDBV are labeled with a fluorophore showing similar characteristics to Cy5.

The probe specific for the target of the Internal Control (IC) is labeled with the fluorophore JOE™ in every Master (1-4).

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® Filovirus Type RT-PCR Kit 2.0 consists of:

- 8 Master reagents
  - Master A and Master B target EBOV
  - Master A and Master B target MARV
  - Master A and Master B target RESTV und TAFV
  - Master A and Master B target SUDV and BDBV
- Internal Control (IC)
- 6 Positive Controls
  - Positive Control EBOV
  - Positive Control MARV
  - Positive Control RESTV
  - Positive Control TAFV
  - Positive Control SUDV
  - Positive Control BDBV
- 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 the Internal Control in one reaction setup.

4.1 Real-Time PCR Instruments

The RealStar® Filovirus Type RT-PCR Kit 2.0 can be used with the following real-time PCR instruments:

- ABI Prism® 7500 SDS (Applied Biosystems)
- QuantStudio™ 5 Real-Time PCR System (Applied Biosystems)
- Rotor-Gene® 6000 (Corbett Research)
- Rotor-Gene® Q5/6 plex Platform (QIAGEN)
- CFX96™ Real-Time PCR Detection System (Bio-Rad)
- CFX96™ Deep Well Real-Time 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® Filovirus Type RT-PCR Kit 2.0.

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 10 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® Filovirus Type RT-PCR Kit 2.0 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	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 Mix 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 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 Master Mix and 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® Filovirus Type RT-PCR Kit 2.0 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	Detector Name	Reporter	Quencher
Zaire ebolavirus specific RNA	1	EBOV	FAM™	(None)
Margburgvirus specific RNA	2	MARV	FAM™	(None)
Reston ebolavirus specific RNA	3	RESTV	FAM™	(None)
Tai Forest ebolavirus specific RNA		TAFV	Cy5	(None)
Sudan ebolavirus specific RNA	4	SUDV	FAM™	(None)
Bundibugyo ebolavirus specific RNA		BDBV	Cy5	(None)
Internal Control	1-4	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	58	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® Filovirus Type RT-PCR Kit 2.0 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			Master	Result Interpretation
FAM™	JOE™	Cy5		
+	+*	N/A	1	EBOV specific RNA detected.
-	+*			No EBOV specific RNA detected.
-	-			RT-PCR inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample.
+	+*	N/A	2	MARV specific RNA detected.
-	+*			No MARV specific RNA detected.
-	-			RT-PCR inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample.
+	+*	-	3	RESTV specific RNA detected.
-	+*	+		TAFV specific RNA detected.
-	+*	-		Neither RESTV nor TAFV specific RNA detected. Sample does not contain detectable amounts of RESTV or TAFV specific RNA.
-	-	-		RT-PCR inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample.

Detection Channel			Master	Result Interpretation
FAM™	JOE™	Cy5		
+	+*	-	4	SUDV specific RNA detected.
-	+*	+		BDBV specific RNA detected.
-	+*	-		Neither SUDV nor BDBV specific RNA detected. Sample does not contain detectable amounts of SUDV or BDBV 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 either in the FAM™ detection channel or in the Cy5 detection channel. A high filovirus RNA load in the sample can lead to reduced or absent Internal Control signals.

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™, ROX™ (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.
i	Note: Information is given to the user that is useful but not essential to the task at hand.
	Version

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always a drop ahead.

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