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RealStar®
Filovirus Screen RT-PCR Kit 1.0

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RealStar®

Filovirus Screen RT-PCR Kit 1.0

For research use only!

(RUO)

REF Product No.: 441003

96 rxns

Store at -25°C ... -15°C

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altona Diagnostics GmbH • Mörkenstraße 12 • D-22767 Hamburg

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The RealStar® Filovirus Screen RT-PCR Kit 1.0 is a reagent system, based on real-time PCR technology, for the qualitative detection of Ebola- and Marburgvirus specific RNA.

1. Kit Components

Lid Color	Blue	Purple	Green	Red	Orange	White
Component	Master A	Master B	Internal Control	Positive Control Ebolavirus	Positive Control Marburgvirus	PCR grade Water
Number of Vials	8	8	1	1	1	1
Volume [µl/Vial]	60	180	1000	500	500	500

2. Storage

- The RealStar® Filovirus Screen 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 -20°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 +4°C should not exceed a period of two hours.
- Protect Master A and Master B from light.

3. Background Information

Ebola- and *Marburgvirus* are genera within the family *Filoviridae*. Genus *Marburgvirus* contains a single species termed *Marburg marburgvirus* (MARV). Genus *Ebolavirus* contains five species: *Bundibugyo ebolavirus* (BEBOV), *Reston ebolavirus* (RESTV), *Sudan ebolavirus* (SEBOV), *Tai Forest ebolavirus* (TAFV) and *Zaire ebolavirus* (ZEBOV) [1].

All known *Ebola-* and *Marburgvirus* species are endemic in Africa except RESTV which is endemic in South-East Asia. Natural hosts of filoviruses are fruit-bats [2] [3]. After transmission to humans, filoviruses can cause a severe hemorrhagic fever with a relatively high mortality rate of 20-90% (depending on the species and strain in the single outbreaks) [4]. The mode of transmission is often difficult to determine. Hunting, slaughtering and consumption of infected wild animals are likely ways of introduction of the virus into the human population. Direct contact to bats has also been shown to be a possible way of infection [5]. Many different mammalian species are susceptible to filovirus infections. In particular chimpanzees and gorillas have been largely affected by *Ebolavirus* epidemics resulting in significant reduction of the great apes populations [6].
Symptoms are rather unspecific at the beginning of the disease including general malaise, fever and pain in different body parts [7]. At the beginning of outbreaks, the disease is therefore often mistaken for Malaria, Typhoid fever or other febrile diseases common in Sub-Saharan Africa.
Infectious virus titer and RNA-titer during acute disease are usually high and the level of viremia is negatively correlated with the outcome of disease [8]. Bleeding and other hemorrhages are also indicators for fatal outcome of Ebola and Marburg fever [7].
Laboratory diagnostics is preferably done using RT-PCR from plasma, serum or even whole blood samples. Serological tests are helpful as supporting diagnostic tools but are not useful for primary diagnosis of the disease. In fact, it has been shown that many patients (especially with fatal outcome) do not develop detectable antibody titers during the course of the disease at all [9].

Several real-time RT-PCR protocols for filovirus detection have been published, but none of them includes an internal amplification control or is able to detect and type *Ebola-* and *Marburgvirus* in a single RT-PCR reaction. The protocol published by Panning and colleagues in 2007 targets the L gene and was shown to be a sensitive and specific assay [10]. Since then, it has been used by several reference laboratories worldwide for filovirus diagnostics. Nevertheless, the latest sequence information available and the occurrence of new Ebola species (BEBOV) showed the need to constantly check and update the existing methods. The 2007 L gene assay has certain weaknesses and therefore a new assay based on the L gene of filoviruses was developed by altona Diagnostics GmbH.

The filovirus L gene, coding for the viral polymerase, contains highly conserved sequence elements. Mutations in regions coding for enzymatically active sites will usually result in loss of function. These mutants will disappear from the viral quasispecies and have no negative impact in the specificity of the RT-PCR based assay. Therefore we decided to use the L gene as target sequence for the RealStar® Filovirus Screen RT-PCR Kit 1.0. The concept of choosing the L gene of RNA-viruses as a target for diagnostic RT-PCRs has been successfully applied in the past for Lassa virus, filoviruses and other RNA-viruses [10–12].

The RealStar® Filovirus Screen RT-PCR Kit 1.0 is recommended as a first line diagnostic test. It is designed to detect all relevant filovirus species. A second line assay is also available from altona Diagnostics GmbH. The RealStar® Filovirus Type RT-PCR Kit 1.0 targets another sequence, the glycoprotein gene, and therefore offers the possibility to generate a confirmatory diagnostic result. Furthermore, the RealStar® Filovirus Type RT-PCR Kit 1.0 allows differentiation of all relevant filoviruses down to the species level.

Suspicion and confirmation of filovirus infections have a great impact on public health and case management. All cases have to be reported immediately to the respective authorities responsible for public health, biosafety and biosecurity (within Germany: Robert Koch Institut, Berlin; and the local “Landesgesundheitsämter”). The diagnostic procedure (e.g. recommended differential diagnosis, possible use of A- and B-sample) has to be discussed with expert reference institutions.

RealStar® Filovirus Screen RT-PCR Kit 1.0

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NOTE

> ⚠ ***Due to the molecular evolution of filoviruses, there is an inherent risk for any PCR based test system that accumulation of mutations over time may lead to false negative results.***

4. Product Description

The RealStar® Filovirus Screen RT-PCR Kit 1.0 is a reagent system, based on real-time PCR technology, for the qualitative detection of filovirus specific RNA.
The reagent system is designed to detect all filovirus species which are relevant human pathogens and Restonvirus. The reagent system includes a heterologous amplification system (Internal Control) to identify possible RT-PCR inhibition and to confirm the integrity of the reagents or the kit.

The test is based on real-time RT-PCR technology, utilizing 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 *Ebolavirus* RNA are labeled with the fluorophore FAM. Probes specific for *Marburgvirus* RNA are labeled with a fluorophore with the same characteristics as Cy5.

The probe specific for the target of the Internal Control (IC) is labeled with the fluorophore JOE. Using probes linked to distinguishable dyes enables the parallel detection and discrimination of *Ebola-* and *Marburgvirus* specific RNA as well as the Internal Control in the corresponding detector channels of the real-time PCR instrument.
Due to the relatively fast molecular evolution of RNA viruses, there is an inherent risk for any RT-PCR based test system that accumulation of mutations over time may lead to false negative results. The assay design is based on the sequence information published in Genebank as of December 2013. Occurrence of new strains and species yet unknown might make an update of primer/probe sets necessary.

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

- Mx 3005P™ QPCR System (Stratagene)
- Versant™ kPCR Molecular System AD (Siemens)
- ABI Prism® 7500 SDS and 7500 Fast SDS (Applied Biosystems)
- LightCycler® 480 Instrument II (Roche)
- Rotor-Gene™ 3000/6000 (Corbett Research)
- Rotor-Gene Q 5/6 plex Platform (QIAGEN)
- CFX96 system/Dx real-time system (BIORAD)

NOTE

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

For additional information and technical support regarding pre-treatment and sample preparation please contact our Technical Support:

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

5. Sample Preparation

Extracted RNA is the starting material for the RealStar® Filovirus Screen 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 nucleic acid extraction kit is recommended:

- QIAamp® Viral RNA Mini Kit (QIAGEN)

If using a spin column based sample preparation procedure including washing buffers containing ethanol, 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 is highly recommended.

NOTE

⚠ ***The use of carrier RNA is crucial for extraction efficiency and stability of the extracted nucleic acid.***

⚠ ***Ethanol is a strong inhibitor in real-time PCR. If your sample preparation system is using washing buffers containing ethanol, you need to make sure to eliminate any traces of ethanol prior to elution of the nucleic acid.***

6. 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 Screen 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, the Master Mix is set up 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, the IC has to be added 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 specimen. The IC should always be added to the specimen/lysis buffer mixture. The volume of the IC which has to be added depends always and only 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 to the specimen/lysis buffer mixture.

NOTE

> ⚠ ***Never add the Internal Control directly to the specimen!***

- If the IC was added during the sample preparation procedure, the Master Mix is set up 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**

7. 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).
- Make sure that each of the Positive Controls and at least one Negative Control is used per run.
- Thoroughly mix the samples and controls with the Master Mix by up and down pipetting.
- Close the 96-well reaction plate with an appropriate optical adhesive film, 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).

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

8. Programming the Real-Time PCR Instruments

For basic information regarding the setup and programming of the different real-time PCR instruments, please refer to the manual of the respective instrument.
For detailed programming instructions regarding the use of the RealStar® Filovirus Screen RT-PCR Kit 1.0 on specific real-time PCR instruments please contact our Technical Support.

8.1 Settings

- Define the following settings:

Settings	
Reaction Volume	30 µl
Ramp Rate	Default
Passive Reference	None

8.2 Fluorescent Detectors (Dyes)

- Define the fluorescent detectors (dyes):

Detection	Detector Name	Reporter	Quencher
Ebolavirus specific RNA	Ebolavirus	FAM	(None)
Marburgvirus specific RNA	Marburgvirus	Cy5	(None)
Internal Control	IC	JOE	(None)

8.3 Temperature Profile and Dye Acquisition

- Define the temperature profile and dye acquisition:

	Stage	Cycle Repeats	Acquisition	Temperature	Time
Reverse Transcription	Hold	1	-	55 °C	20:00 min
Denaturation	Hold	1	-	95 °C	2:00 min
Amplification	Cycling	45	-	95 °C	0:15 min
			√	58 °C	0:45 min
			-	72 °C	0:15 min

9. Data Analysis

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

For detailed instructions regarding data analysis of the RealStar® Filovirus Screen RT-PCR Kit 1.0 on different real-time PCR instruments please contact our Technical Support.

10. Interpretation of Results

Sample ID	FAM Detection Channel	Cy5 Detection Channel	JOE Detection Channel	Result Interpretation
A	POSITIVE	NEGATIVE	POSITIVE*	Ebolavirus specific RNA detected.
B	NEGATIVE	POSITIVE	POSITIVE*	Marburgvirus specific RNA detected.
C	NEGATIVE	NEGATIVE	POSITIVE	Neither Ebola- nor Marburgvirus specific RNA detected. Sample does not contain detectable amounts of Ebola- or Marburgvirus specific RNA.
D	NEGATIVE	NEGATIVE	NEGATIVE	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. High filovirus load in the sample can lead to reduced or absent Internal Control signals.

11. Technical Assistance

For customer support, please contact our Technical Support:

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

12. Trademarks and Disclaimers

RealStar® (altona Diagnostics GmbH); Mx 3005P™ (Stratagene); ABI Prism® (Applied Biosystems); LightCycler® (Roche); Rotor-Gene™, QIAamp® (QIAGEN); VERSANT™ (Siemens); CFX96™ (BIO-RAD).

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For research use only! Not for use in diagnostic procedures.

Not available in all countries.

13. Explanation of Symbols

Product number

Batch code


Contains sufficient for “n” tests/reactions (rxns)


Temperature limitation


Version


Use until


Caution


Consult instructions for use


Manufacturer

Notes