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Instructions for Use

RealStar®
MERS-CoV RT-PCR Kit 1.0

01/2017 EN

RealStar®

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MERS-CoV RT-PCR Kit 1.0

For research use only!

(RUO)

INS-391000-EN-S02

REF 391002

Σ 48

01 2017

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Content

1. Application

The RealStar® MERS-CoV RT-PCR Kit 1.0 is a reagent system, based on real-time PCR technology, for the qualitative detection of Middle East respiratory syndrome coronavirus (MERS-CoV) 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 Target orf1a	4	60
Purple	Master B Target orf1a	4	120
Blue	Master A Target upE	4	60
Purple	Master B Target upE	4	120
Red	Positive Control	1	250
Green	Internal Control	1	1000
White	Water (PCR grade)	1	500

3. Storage

- The RealStar® MERS-CoV 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® MERS-CoV RT-PCR Kit 1.0 is a reagent system system, based on real-time PCR technology, for the qualitative detection of Middle East respiratory syndrome coronavirus (MERS-CoV) specific RNA.

The RealStar® MERS-CoV RT-PCR Kit 1.0 consists of two independent assays, one targeting a region upstream of the E gene (*upE*) and the other targeting open reading frame 1a (*orf1a*) of the MERS-CoV genome. World Health Organization (WHO) recommends the use of two independent PCR assays for confirmation of MERS-CoV cases **[5]**.

Both assays include 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.

In both assays, probes specific for MERS-CoV RNA are labeled with the fluorophore FAM™. The probe specific for the target of the Internal Control (IC) is labelled with the fluorophore JOE™. Using probes linked to distinguishable dyes enables the parallel detection of MERS-CoV specific RNA and the Internal Control in the corresponding detector channels of the real-time PCR instrument.

The oligonucleotides included in the two assays were previously published by Victor Corman et al. 2012a **[6]** and 2012 b **[7]**. One RT-PCR assay targets the *orf1a* (Master A with blue caps, corresponding Master B with purple caps) and the other targets a region upstream of the E-gene (*upE*) (Master A with blue caps, corresponding Master B with purple caps).

Following the WHO-case definition (http://www.who.int/), laboratory confirmation requires two positive RT-PCR results on independent targets. By parallel testing of samples with the *upE*- and the *orf1a*-assay, which are both included in the RealStar® MERS-CoV RT-PCR Kit 1.0, the WHO requirements for laboratory MERS-CoV case confirmation can be fulfilled.

Due to the molecular assembly and possible evolution of MERS-CoV, there is an inherent risk for any RT-PCR based test system that accumulation of mutations over time may lead to false negative results. By including two assays targeting two different regions of the genome, the risk is significantly reduced. In case only one of the two assays included in the kit gives a positive result, the sample should be retested. **Furthermore, positive samples should be sent to the national reference laboratory for confirmatory testing**.

Nevertheless, in case the circulating strains evolve and accumulate mutations an update of the primer/probe sets might be necessary.

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® MERS-CoV RT-PCR Kit 1.0 consists of:

- Four Master reagents
  - (Master A and Master B target *orf1a*)
  - (Master A and Master B target *upE*)
- Internal Control (IC)
- Positive Control
- 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 target detection of MERS-CoV specific RNA and Internal Control in one reaction setup.

4.1 Real-Time PCR Instruments

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

- m2000rt (Abbott Diagnostics)
- Mx 3005P™ QPCR System (Stratagene)
- VERSANT® kPCR Molecular System AD (Siemens Healthcare)
- ABI Prism® 7500 SDS (Applied Biosystems)
- ABI Prism® 7500 Fast SDS (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 PCR Detection System (Bio-Rad)
- LightCycler® 480 Instrument II (Roche)

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® MERS-CoV 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.***

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® MERS-CoV 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 (*orf1a* or *upE*)	5 µl	60 µl
Master B (*orf1a* or *upE*)	10 µl	120 µl
Internal Control	1 µl	12 µl
Volume Master Mix	**16 µl**	**192 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 (*orf1a* or *upE*)	5 µl	60 µl
Master B (*orf1a* or *upE*)	10 µl	120 µl
Volume Master Mix	**15 µl**	**180 µl**

CAUTION	
	***If the IC (Internal Control) was added during the sample preparation procedure, at least the negative control must include the IC.***
	***No matter which method/system is used for nucleic acid extraction, never add the IC directly to the sample.***

5.3 Reaction Setup

- ► Pipette 15 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	15 µl
Sample or Control	10 µl
Total Volume	**25 µl**

- ► Make sure that at least one Positive and 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 1000 x g (~ 3000 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® MERS-CoV 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	25 µl
Ramp Rate	Default
Passive Reference	None

6.2 Fluorescence Detectors (Dyes)

- ► Define the fluorescence detectors (dyes):

Target	Detector Name	Reporter	Quencher
MERS-CoV (*orf1a*) specific RNA	*orf1a*	FAM™	(None)
MERS-CoV (*upE*) specific RNA	*upE*	FAM™	(None)
Internal Control	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® MERS-CoV 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™ upE	JOE™ upE	FAM™ orf1a	JOE™ orf1a	
+	+*	+	+*	MERS-CoV upE and orf1a specific RNA detected. Send positive samples to the national reference laboratory for confirmatory testing
+	+*	-	+	MERS-CoV upE specific RNA detected. No MERS-CoV orf1a specific RNA detected. Repeat testing. Send sample to the national reference laboratory for confirmatory testing.
-	+	+	+*	MERS-CoV orf1a specific RNA detected. No MERS-CoV upE specific RNA detected. Repeat testing. Send sample to the national reference laboratory for confirmatory testing.
-	+	-	+	Neither MERS-CoV upE nor MERS-CoV orf1a specific RNA detected. The sample does not contain detectable amounts of MERS-CoV 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 MERS-CoV RNA load in the sample can lead to a reduced or absent Internal Control signal.

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

RealStar® (altona Diagnostics); ABI Prism® (Applied Biosystems); ATCC® (American Type Culture Collection); CFX96™ (Bio-Rad); Cy® (GE Healthcare); FAM™, JOE™, ROX™ (Life Technologies); LightCycler® (Roche); Maxwell® (Promega); Mx 3005P™ (Stratagene); NucliSENS®, easyMag® (bioMérieux); Rotor-Gene®, QIAamp®, QIAsymphony® (QIAGEN); VERSANT® (Siemens Healthcare).

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

10. Explanation of Symbols

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

always a drop ahead.

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