

Instructions for Use

RealStar[®] SARS-CoV-2 RT-PCR Kit 1.0

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RealStar[®]

SARS-CoV-2 RT-PCR Kit 1.0

For research use only!

(RUO)



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1. Application

The RealStar® SARS-CoV-2 RT-PCR Kit 1.0 is a reagent system, based on real-time PCR technology, for the qualitative detection of lineage B-beta coronavirus (B-βCoV) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) 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	240
Purple	Master B	8	720
Green	Internal Control	4	1000
Red	Positive Control*	2	250
White	Water (PCR grade)	2	500

* The Positive Control contains both targets, lineage B-βCoV and SARS-CoV-2

3. Storage

- The RealStar® SARS-CoV-2 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. Background Information

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a positive sense, single stranded RNA virus belonging to the family Coronaviridae, genus betacoronavirus, subgenus lineage B.

SARS-CoV-2 emerged in the Wuhan region of China in December 2019 and has spread worldwide within 2 months. The virus was initially termed as 2019-nCoV (novel Coronavirus) and renamed as SARS-CoV-2 by the "International Committee on Taxonomy of Viruses", on 11.02.2020. At the same time the WHO named the disease, caused by SARS-CoV-2, COVID-19. Considering the rapid escalation and propagation of COVID-19 worldwide, the WHO characterized the outbreak as a pandemic on 12.03.2020.

SARS-CoV-2 is highly contagious and transmitted via aerosols and droplets and causes acute respiratory infections with flu-like symptoms. Mainly, but not exclusively, in elderly people and persons with pre-existing illness, infection with SARS-CoV-2 can lead to severe and life-threatening disease. Cases of asymptomatic infection, mild illness, severe illness, and deaths have been reported.

5. Product Description

The RealStar® SARS-CoV-2 RT-PCR Kit 1.0 is a reagent system, based on real-time PCR technology, for the qualitative detection of lineage B-beta coronavirus (B-βCoV) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) 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 labeled with fluorescent reporter and quencher dyes.

The probe specific for lineage B-βCoV (target E gene) RNA is labeled with the fluorophore FAM™ whereas the probe specific for SARS-CoV-2 (target S gene) RNA is labeled with the fluorophore Cy5. The probe specific for Internal Control (IC) is labeled with the fluorophore JOE™.

Using probes linked to distinguishable dyes enables the parallel detection of lineage B-βCoV specific RNA and SARS-CoV-2 specific RNA as well as the detection of 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 labeled probes

The RealStar® SARS-CoV-2 RT-PCR Kit 1.0 consists of:

- Master A
- Master B
- Internal Control
- Positive Control (B-βCoV, SARS-CoV-2)
- Water (PCR grade)

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 lineage B-βCoV (target E gene) specific RNA, SARS-CoV-2 (target S gene) specific RNA and the Internal Control in one reaction setup.

5.1 Real-Time PCR Instruments

The RealStar® SARS-CoV-2 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)
- Mx 3005P™ QPCR System (Stratagene)
- Rotor-Gene® 6000 (Corbett Research)
- Rotor-Gene® Q5/6 plex Platform (QIAGEN)
- VERSANT® kPCR Molecular System AD (Siemens Healthcare Diagnostics)

NOTE

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

6. Procedure

6.1 Sample Preparation

Extracted RNA is the starting material for the RealStar® SARS-CoV-2 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:

- AltoStar® Automation System AM16 (Altona Diagnostics)
- 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 9. Technical Assistance).

6.2 Master Mix Setup

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

The RealStar® SARS-CoV-2 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	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.

6.3 Reaction Setup

- ▶ Pipette 20 μ l of the Master Mix into each required well of an appropriate optical 96-well reaction plate or an appropriate optical reaction tube.
- ▶ Add 10 μ l of the sample (eluate from the nucleic acid extraction) or 10 μ l of the controls (Positive or Negative Control).

Reaction Setup	
Master Mix	20 μ l
Sample or Control	10 μ l
Total Volume	30 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 1,000 x g (~ 3,000 rpm).

7. 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® SARS-CoV-2 RT-PCR Kit 1.0 on specific real-time PCR instruments please contact our Technical Support (see chapter 9. Technical Assistance).

7.1 Settings

- Define the following settings:

Settings	
Reaction Volume	30 µl
Ramp Rate	Default
Passive Reference	ROX™

7.2 Fluorescence Detectors (Dyes)

- Define the fluorescence detectors (dyes):

Target	Detector Name	Reporter	Quencher
Lineage B-βCoV specific RNA	E gene	FAM™	(None)
SARS-CoV-2 specific RNA	S gene	Cy5	(None)
Internal Control	IC	JOE™	(None)

7.3 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

8. 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® SARS-CoV-2 RT-PCR Kit 1.0 on different real-time PCR instruments please contact our Technical Support (see chapter 9. Technical Assistance).

8.1 Interpretation of Results

8.1.1 Qualitative Analysis

Detection Channel			Result Interpretation
FAM™ (E gene)	Cy5 (S gene)	JOE™ (Internal Control)	
+	+	+	Lineage B-βCoV and SARS-CoV-2 specific RNA detected. Positive for SARS-CoV-2.
+	-	+	Only lineage B-βCoV specific RNA detected. Presumptive positive for SARS-CoV-2. ^{1,2}
-	+	+	Only SARS-CoV-2 specific RNA detected. Positive for SARS-CoV-2. ¹
-	-	+	Neither lineage B-βCoV nor SARS-CoV-2 specific RNA detected. The sample does not contain detectable amounts of SARS-CoV-2 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 lineage B-βCoV (target E gene) and/or SARS-CoV-2 (target S gene) RNA load in the sample can lead to reduced or absent Internal Control signals.

¹ Detection in only one of the two respective detection channels for E gene and S gene might be due to low viral RNA concentration close to the limit of detection or due to mutation of one of the two target sequences.

² Sample may be re-tested by repeating the extraction and RT-PCR. If the repeated result remains presumptive positive then additional confirmatory testing may be conducted.

9. Technical Assistance

For customer support, please contact our Technical Support:

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

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

10. Trademarks and Disclaimers














AltoStar®, RealStar® (altona Diagnostics); ABI Prism® (Applied Biosystems); EasyMAG®, NucliSENS® (bioMérieux); CFX96™ (Bio-Rad); JOE™ (Life Technologies); Maxwell® (Promega); Rotor-Gene®, QIAamp®, QIASymphony® (QIAGEN); LightCycler® (Roche); VERSANT® (Siemens Healthcare); FAM™, ROX™ (Thermo Fisher Scientific).



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11. Explanation of Symbols

Symbol	Explanation
	For Research Use Only
	Batch code
	Cap color
	Catalogue number
	Content
	Number
	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, if 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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