altona DIAGNOSTICS

Instructions for Use

RealStar®
SARS-CoV-2 RT-PCR Kit 1.0

01/2021 EN

RealStar®

RealStar®

SARS-CoV-2 RT-PCR Kit 1.0

For use with

CFX96™ Dx System (Bio-Rad)

CFX96™ Deep Well Dx System (Bio-Rad)

QuantStudio™ 5 Real-Time PCR System (Applied Biosystems)

ABI Prism® 7500 SDS (Applied Biosystems)

LightCycler® 480 Instrument II (Roche)

Rotor-Gene® Q5/6 plex Platform (QIAGEN)

CE

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Content

1. Intended Use

The RealStar® SARS-CoV-2 RT-PCR Kit 1.0 is an *in vitro* diagnostic test, based on real-time PCR technology, for the qualitative detection of lineage B-beta coronavirus (lineage B-βCoV) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) specific RNA.

It is intended to be used as an aid for diagnosis in individuals with signs and symptoms of coronavirus disease 2019 (COVID-2019) in conjunction with clinical and epidemiological risk factors.

The RealStar® SARS-CoV-2 RT-PCR Kit 1.0 is intended to be used by qualified personnel in appropriately equipped laboratories following the guidelines on laboratory biosafety.

2. Kit Components

Lid Color	Component	Number of Vials	Volume [µl/Vial]
Blue	Master A	8	240
Purple	Master B	8	720
Red	Positive Control*	2	250
Green	Internal Control	4	1000
White	Water (PCR grade)	2	500

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

CAUTION	
	***Before first use check the product and its components for completeness with respect to number, type and filling. Do not use a defective or incomplete product, performance could be compromised.***

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, Internal Control and Positive Control (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.

CAUTION	
	***Improper storage conditions may lead to a compromised product performance.***

CAUTION	
	***Do not exceed thaw-freeze-sequence and handling durations as specified in these Instructions for Use.***

CAUTION	
	***Do not use product components beyond the expiration date printed on the component label.***

4. Material and Devices required but not provided

- Appropriate real-time PCR instrument (see chapter 6.1 Real-Time PCR Instruments)
- Appropriate nucleic acid extraction system or kit (see chapter 8.1 Sample Preparation)
- Desktop centrifuge with a rotor for 2 ml reaction tubes
- Centrifuge with a rotor for microtiter plates, if using 96 well reaction plates
- Vortex mixer
- Appropriate 96 well reaction plates or reaction tubes with corresponding (optical) closing material
- Pipettes (adjustable)
- Pipette tips with filters (disposable)
- Powder-free gloves (disposable)

NOTE

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

NOTE

It is highly recommended to use the 72-well rotor with the appropriate 0.1 ml reaction tubes, if using the Rotor-Gene® Q 5/6 plex (QIAGEN).

5. 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.

6. Product Description

The RealStar® SARS-CoV-2 RT-PCR Kit 1.0 is an *in vitro* diagnostic test, based on real-time PCR technology, for the qualitative detection of lineage B-beta coronavirus (lineage 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
- Positive Control (B-βCoV, SARS-CoV-2)
- Internal Control
- 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.

6.1 Real-Time PCR Instruments

The RealStar® SARS-CoV-2 RT-PCR Kit 1.0 was developed and validated to be used with the following real-time PCR instruments:

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

6.2 Sample Types, Handling and Storage

The RealStar® SARS-CoV-2 RT-PCR Kit 1.0 has been validated for use with the following sample type:

- Human respiratory swabs collected in Universal Transport Medium™ (UTM®)

The RealStar® SARS-CoV-2 RT-PCR Kit 1.0 has been validated using the AltoStar® Purification Kit 1.5 on the AltoStar® Automation System AM16 for nucleic acid extraction and purification.

Commercially available dacron fiber- or polyester-tipped swabs with plastic shafts have to be used for sample collection. Dry swabs must be resuspended in universal transport medium (e.g. UTM® from Copan). Calcium alginate swabs, swabs with wooden shafts and/or cotton tips as well as swabs collected in agar gel must not be used. Transport should occur following the local and national instructions for the transport of biological material.

Before use respiratory swabs resuspended in UTM® should not be stored for more than 48 hours at room temperature (+20 °C to +25 °C), 5 days at +2 °C to +8 °C or 2 months at -25 °C to -15 °C.

CAUTION

Always treat samples as infectious and (bio-)hazardous in accordance with safe laboratory procedures. For sample material spills promptly use an appropriate disinfectant. Handle contaminated materials as biohazardous.

NOTE

Frozen storage of samples does not compromise kit performance. When working with frozen samples, make sure samples are completely thawed and properly mixed before use.

NOTE

Do not use calcium alginate swabs, since this might lead to incorrect or invalid results due to PCR inhibition.

7. Warnings, Precautions and Limitations

- Before first use check the product and its components for completeness with respect to number, type and filling. Do not use a defective or incomplete product, performance could be compromised.
- Do not use other sample types! The use of other sample types may compromise the product performance.
- The presence of PCR inhibitors may cause false negative or invalid results.
- In case the sample contains other pathogens than SARS-CoV-2 competition with the target amplification or cross-reactivities may occur.
- Improper storage conditions may lead to a compromised product performance.
- A lack of centrifugation of the product components after thawing could lead to contamination of the components with reagent residues in the lids and as consequence to a compromised product performance.
- Do not exceed thaw-freeze-sequence and handling durations as specified in these Instructions for Use.
- Do not use product components beyond the expiration date printed on the component label.
- Improper handling of product components and samples may lead to contamination causing incorrect IVD examination results.
    - Do not interchange vial or bottle caps, as cross-contamination may occur.
    - To minimize the risk of carryover contamination store positive and/or potentially positive material separated from the kit components.
    - Use separated working areas for sample preparation/reaction setup and amplification/detection activities.
    - Always wear disposable gloves.
    - Do not open the PCR plates or tubes post amplification to avoid contamination with amplicons.
- Storage of eluates under wrong conditions may lead to degradation of the SARS-CoV-2 target sequences.

- Do not exceed the PCR Mix storage time. This could lead to a compromised product performance.
- Always treat samples as infectious and (bio-)hazardous in accordance with safe laboratory procedures. For sample material spills promptly use an appropriate disinfectant. Handle contaminated materials as biohazardous.
- Dispose of hazardous and biological waste only in compliance with local and national regulations to avoid environmental contamination.
- As with any diagnostic test, results should be interpreted in consideration of all clinical and laboratory findings.
- Potential mutations within the target regions of the SARS-CoV-2 genome covered by the primers and/or probes used in the kit may result in failure to detect the presence of the pathogen.
- 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.
- The use of carrier RNA is crucial for extraction efficiency and stability of the extracted nucleic acid.
- This assay must not be used on the specimen directly. Appropriate nucleic acid extraction methods have to be conducted prior to using this assay.
- Strict compliance with the Instructions for Use is required for optimal results.
- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.
- Good laboratory practice is essential for proper performance of this assay. Extreme care should be taken to preserve the purity of the components of the kit and reaction setups. All reagents should be closely monitored for impurity and contamination. Any suspicious reagents should be discarded.
- Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test.
- The E gene assay (FAM™ channel) does detect lineage B-betacoronavirus specific RNA including SARS coronavirus and several bat coronaviruses. Isolated signals with the E gene assay could indicate the presence of SARS coronavirus or bat coronaviruses.

8. Procedure

CAUTION	
	***Improper handling of product components and samples may lead to contamination causing incorrect IVD examination results.*** ***- Do not interchange vial or bottle caps, as cross-contamination may occur.*** ***- To minimize the risk of carryover contamination store positive and/or potentially positive material separated from the kit components.*** ***- Use separated working areas for sample preparation/reaction setup and amplification/detection activities.*** ***- Always wear disposable gloves.*** ***- Do not open the PCR plates or tubes post amplification to avoid contamination with amplicons.***

8.1 Sample Preparation

Extracted RNA is the starting material for the RealStar® SARS-CoV-2 RT-PCR Kit 1.0.

The RealStar® SARS-CoV-2 RT-PCR Kit 1.0 was validated with human respiratory swabs using the AltoStar® Automation System AM16 in combination with the AltoStar® Purification Kit 1.5.

Alternative nucleic acid extraction systems and kits (see below) might also be appropriate. The suitability of the nucleic acid extraction procedure for use with RealStar® SARS-CoV-2 RT-PCR Kit 1.0 has to be validated by the user.

- 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)

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.

After completion of the extraction procedure the eluates in the unsealed eluate plate are stable at room temperature (max. +30 °C) for a total of 6 hours. The eluates in a sealed eluate plate can be stored at +2 °C to +8 °C for up to 24 hours before the start of a PCR reaction setup.

CAUTION	
	***Do not use other sample types! The use of other sample types may compromise the product performance.***

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.***

CAUTION	
	***Always treat samples as infectious and (bio-)hazardous in accordance with safe laboratory procedures. For sample material spills promptly use an appropriate disinfectant. Handle contaminated materials as biohazardous.***

CAUTION	
	***The presence of PCR inhibitors may cause false negative or invalid results.***

CAUTION	
	***This assay must not be used on the specimen directly. Appropriate nucleic acid extraction methods have to be conducted prior to using this assay.***

CAUTION	
	***Dispose of hazardous and biological waste only in compliance with local and national regulations to avoid environmental contamination.***

CAUTION	
	***Storage of eluates under wrong conditions may lead to degradation of the lineage B-βCoV and SARS-CoV-2 target sequences.***

For additional information and technical support regarding pre-treatment and sample preparation please contact our Technical Support (see chapter 13. Technical Assistance).

8.2 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 specimen. The IC should always be added to the specimen/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 specimen/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

A lack of centrifugation of the product components after thawing could lead to contamination of the components with reagent residues in the lids and as consequence to a compromised product performance.

NOTE

If the IC (Internal Control) was added during the sample preparation procedure, at least the negative control must include the IC.

NOTE

No matter which method/system is used for nucleic acid extraction, never add the IC directly to the specimen.

8.3 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 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).

After completion of the PCR Reaction Setup the PCR Mix is stable at room temperature (max. +30 °C) for 30 minutes.

CAUTION

Do not exceed the PCR Mix storage time. This could lead to a compromised product performance.

9. 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 13. Technical Assistance).

9.1 Settings

► Define the following settings:

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

9.2 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)

9.3 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	55	00:45
			-	72	00:15

10. 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 13. Technical Assistance).

10.1 Validity of Diagnostic Test Runs

10.1.1 Valid Diagnostic Test Run

A diagnostic test run is **valid**, if the following control conditions are met:

Control ID	Detection Channel		
	FAM™	Cy5	JOE™
Positive Control [lineage B-βCoV and SARS-CoV-2]	+	+	+/-*
Negative Control	-	-	+

* The presence or absence of a signal in the JOE™ channel is not relevant for the validity of the test run.

10.1.2 Invalid Diagnostic Test Run

A diagnostic test run is **invalid**, (i) if the run has not been completed or (ii) if any of the control conditions for a **valid** diagnostic test run are not met.

In case of an **invalid** diagnostic test run, repeat testing by using the remaining purified nucleic acids or start from the original samples again.

10.2 Interpretation of Results

CAUTION	
	***As with any diagnostic test, results should be interpreted in consideration of all clinical and laboratory findings.***

10.2.1 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.

11. Performance Evaluation

The analytical performance evaluation of the RealStar® SARS-CoV-2 RT-PCR Kit 1.0 was done using heat inactivated SARS-CoV-2 cell culture supernatant (*BetaCoV/Munich/ChVir984/2020*) provided by the Institute of Virology, Charité, Berlin; Germany.

11.1 Analytical Sensitivity

The analytical sensitivity was determined by analysis of dilution series of heat inactivated SARS-CoV-2 cell culture supernatant (*BetaCoV/Munich/ChVir984/2020* provided by the Institute of Virology, Charité, Berlin; Germany) diluted in Universal Transport Medium™ (UTM®, Copan) containing simulated nasal matrix [5 % w/v Mucin, 5 % v/v blood, 0.8 % v/v NaCl (95 % saline) and 0.00002 % w/v human genomic DNA (510k Submission for BD MAX™ MRSA XT assay; accession number: K133605)].

Each dilution was tested in 8 replicates on 3 different days (total n = 24 per dilution) using combinations of 3 RealStar® SARS-CoV-2 RT-PCR Kit 1.0 lots, 3 AltoStar® Purification Kit 1.5 lots and 3 AltoStar® Internal Control 1.5 lots. Runs were performed using 3 different AltoStar® Automation System AM16 and CFX96™ Deep Well Dx System (Bio-Rad).

Data from all runs were combined and a probit analysis was performed to determine the 95 % LoD value.

Table 2: RT-PCR results used for the calculation of the analytical sensitivity with respect to the detection of SARS-CoV-2 specific RNA (E gene)

Input Conc. [PFU/ml]	Number of Replicates	Number of Positives	Hit Rate [%]
1.00E+00	24	24	100
3.16E-01	24	24	100
1.00E-01	24	24	100
3.16E-02	24	24	100
1.00E-02	24	21	88
3.16E-03	24	12	50
1.00E-03	24	4	17
3.16E-04	24	1	4
1.00E-04	24	2	8

The analytical sensitivity of the RealStar® SARS-CoV-2 RT-PCR Kit 1.0 for SARS-CoV-2 (E gene) was determined by probit analysis. For the detection of SARS-CoV-2 RNA (E gene), the analytical sensitivity is 0.025 PFU/ml [95 % confidence interval (CI): 0.014 - 0.060 PFU/ml].

Table 3: RT-PCR results used for the calculation of the analytical sensitivity with respect to the detection of SARS-CoV-2 specific RNA (S gene)

Input Conc. [PFU/ml]	Number of Replicates	Number of Positives	Hit Rate [%]
1.00E+00	24	24	100
3.16E-01	24	24	100
1.00E-01	24	24	100
3.16E-02	24	24	100
1.00E-02	24	23	96
3.16E-03	24	15	63
1.00E-03	24	4	17
3.16E-04	24	2	8
1.00E-04	24	1	4

The analytical sensitivity of the RealStar® SARS-CoV-2 RT-PCR Kit 1.0 for SARS-CoV-2 (S gene) was determined by probit analysis. For the detection of SARS-CoV-2 RNA (S gene), the analytical sensitivity is 0.014 PFU/ml [95 % confidence interval (CI): 0.008 - 0.032 PFU/ml].

11.2 Analytical Specificity

The analytical specificity of the RealStar® SARS-CoV-2 RT-PCR Kit 1.0 is ensured by the thorough selection of the oligonucleotides (primers and probes). The oligonucleotides were checked by sequence comparison analysis against publicly available sequences to ensure that all relevant lineage B-βCoV (target E gene) and SARS-CoV-2 (target S gene) genotypes will be detected.

11.2.1 Inclusivity

Inclusivity of the RealStar® SARS-CoV-2 RT-PCR Kit 1.0 was evaluated for different isolates of SARS-CoV-2 by wet testing. The results are shown in Table 4.

Table 4: Inclusivity (wet testing) RealStar® SARS-CoV-2 RT-PCR Kit 1.0

SARS-CoV-2 Strain/Isolate	Source/Sample Type	Concentration
*BetaCoV/Munich/ ChVir984/2020**	Institute of Virology; Charité Berlin; Germany/ Heat inactivated cell culture supernatant	1.00E+04 copies/µl
2019-nCoV/Italy-INMI1	European Virus Archive Global/RNA	1.00E+06 copies/µl

* The strain *BetaCoV/Munich/ChVir984/2020* was used for the determination of the LoD and the evaluation of the clinical performance of the RealStar® SARS-CoV-2 RT-PCR Kit 1.0.

Table 5: Inclusivity (*In silico* analysis for 155,031 whole genome sequences of SARS-CoV-2 published via GISAID e.V. (www.gisaid.org) and 36,630 whole genome sequences published via the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov) as of November 5, 2020 for the E gene and the S gene target): RealStar® SARS-CoV-2 RT-PCR Kit 1.0

191,661 Whole Genome Sequences		Homology	Comment
E gene	Forward Primer	191,553 sequences: 100 %	106 sequences: 96.2 % (1 mismatch) 2 sequences: 92.3 % (2 mismatches)
	Reverse Primer	191,602 sequences: 100 %	58 sequences: 95.5 % (1 mismatch) 1 sequence: 90.9 % (2 mismatches)
	Probe	191,539 sequences: 100 %	122 sequences: 95.7 % (1 mismatch)
S gene	Forward Primer	191,419 sequences: 100 %	239 sequences: 95.2 % (1 mismatch) 3 sequences: 90.5 % (2 mismatches)
	Reverse Primer	190,996 sequences: 100 %	662 sequences: 95.5 % (1 mismatch) 3 sequences: 90.1 % (2 mismatches)
	Probe	190,534 sequences: 100 %	1,120 sequences: 96.3 % (1 mismatch) 6 sequences: 92.6 % (2 mismatches) 1 sequence: 85.2 %* (4 mismatches)

* The sequence (accession ID EPI_ISL_415593, GISAID) showed 4 mismatches in the S gene probe binding site. This sequence was published on March 10, 2020 originating from Washington, USA. Since then none of the published sequences showed that many mismatches again. The sequence was commented by the authors “Caution. Stretches of NNNs (1.74 % of overall sequence)”, indicating not ideal sequencing quality, the impact on the S gene specific oligonucleotides has therefore not been investigated.

Depending on the position, mutation events leading to ≤ 2 mismatch/es in a single oligonucleotide sequence are very unlikely to have any significant negative effect on the performance of the assay. All such sequences (≤ 2 mismatch/es) tested in wet lab experiments in the cause of the post market surveillance activities for the RealStar® SARS-CoV-2 RT-PCR Kit 1.0 so far confirmed that the performance was not affected by such mutations. With the exception of one unique sequence none of the other analyzed sequences showed mismatches in more than one oligonucleotide and none of the mismatching sequences showed mismatches with both specific detection systems (E gene and S gene), hence reactivity of the specific oligonucleotides included in the RealStar® SARS-CoV-2 RT-PCR Kit 1.0 is not expected to be affected.

11.2.2 Cross Reactivity

The analytical specificity of the RealStar® SARS-CoV-2 RT-PCR Kit 1.0 with respect to cross reactivity with other pathogens than SARS-CoV-2 was evaluated by testing viruses related to SARS-CoV-2, pathogens causing similar symptoms as an infection with SARS-CoV-2 and pathogens likely to be present in patients suffering from a SARS-CoV-2 infection.

With the exception of SARS-coronavirus* the RealStar® SARS-CoV-2 RT-PCR Kit 1.0 did not cross-react with any of the following pathogens:

- Human coronavirus 229E
- Human coronavirus OC43
- Human coronavirus NL63
- MERS-coronavirus
- Adenovirus
- Human Metapneumovirus (hMPV)
- Parainfluenza virus 1
- Parainfluenza virus 2
- Parainfluenza virus 3
- Parainfluenza virus 4
- Influenza A virus
- Influenza B virus
- Enterovirus
- Respiratory syncytial virus A
- Repiratory syncytial virus B
- Rhinovirus
- *Chlamydia pneumoniae*
- *Haemophilus influenzae*

- *Legionella pneumophila*
- *Mycobacterium tuberculosis*
- *Streptococcus pneumoniae*
- *Streptococcus pyogenes*
- *Bordetella pertussis*
- *Mycoplasma pneumoniae*
- *Pneumocystis jirovecii* (PJP)
- *Candida albicans*
- *Pseudomonas aeruginosa*
- *Staphylococcus epidermidis*
- *Streptococcus salivarius*

* A positive result is obtained for SARS-coronavirus with the RealStar® SARS-CoV-2 RT-PCR Kit 1.0 in the FAM™ channel, since the E gene target is not SARS-CoV-2 specific, but detects all lineage B-betacoronaviruses including SARS-coronavirus.

CAUTION

In case the sample contains other pathogens than SARS-CoV-2 competition with the target amplification or cross-reactivities may occur.

11.3 Precision

Precision of the RealStar® SARS-CoV-2 RT-PCR Kit 1.0 was determined as intra-assay variability (variability within one experiment), inter-assay variability (variability between different experiments) and inter-lot variability (variability between different production lots). Total variability was calculated by combining the 3 analyses.

The variability data are expressed in terms of standard deviation and coefficient of variation based on threshold cycle (C_t) - values. At least 4 replicates per sample were analysed for intra-assay variability, inter-assay and inter-lot variability.

Table 6: Precision data (CV % [C_t values]) for SARS-CoV-2 high positive UTM® samples

	SARS-CoV-2 High Positive Sample [C_t in the FAM™ channel, target E gene]	SARS-CoV-2 High Positive Sample [C_t in the Cy5 channel, target S gene]
Intra-Assay Variability	0.15 - 0.61	0.02 - 0.34
Inter-Assay Variability	1.80 - 2.10	1.53 - 1.64
Inter-Lot Variability	0.44	0.41
Total Variability	1.83	1.22

All samples tested at 3 x LoD (low positive samples) were detected positive for SARS-CoV-2 (E gene and S gene).

Table 7: Precision data (CV % [C_t values]) for the Internal Control in SARS-CoV-2 negative UTM® samples

	Internal Control
Intra-Assay Variability	0.12 - 0.49
Inter-Assay Variability	0.36 - 1.33
Inter-Lot Variability	0.39
Total Variability	**1.02**

11.4 Diagnostic Evaluation

The RealStar® SARS-CoV-2 RT-PCR Kit 1.0 was evaluated in a comparative study with the CE-marked Bosphore Novel Coronavirus (2019-nCoV) Detection Kit (Anatolia Geneworks). Retrospectively, 110 respiratory swab samples from routine SARS-CoV-2 monitoring were tested in parallel using the Bosphore Novel Coronavirus (2019-nCoV) Detection Kit in combination with the *m*Sample Preparation Systems RNA (Abbott) and the *m*2000sp Instrument (Abbott) and the RealStar® SARS-CoV-2 RT-PCR Kit 1.0 in combination with the AltoStar® Purification Kit 1.5 and the AltoStar® Internal Control 1.5 on the AltoStar® Automation System AM16 and the CFX96™ Deep Well Dx System (Bio-Rad). For the qualitative analysis all samples with an invalid result for one or both assays were excluded. Results for the remaining 104 samples are shown in Table 8.

Table 8: Results of the evaluation of the diagnostic sensitivity and specificty for SARS-CoV-2 in respiratory swab samples

		Bosphore Novel Coronavirus (2019-nCoV) Detection Kit (Anatolia Geneworks)	
		POSITIVE	NEGATIVE
RealStar® SARS-CoV-2 RT-PCR Kit 1.0	POSITIVE	51	2
	NEGATIVE	0	51

The diagnostic sensitivity and specificity of the RealStar® SARS-CoV-2 RT-PCR Kit 1.0 compared to the Bosphore Novel Coronavirus (2019-nCoV) Detection Kit were 100 % and 96 %, respectively.

12. Quality Control

In accordance with the altona Diagnostics GmbH ISO EN 13485-certified Quality Management System, each lot of RealStar® SARS-CoV-2 RT-PCR Kit 1.0 is tested against predetermined specifications to ensure consistent product quality.

13. Technical Assistance

For customer support, please contact our Technical Support:

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

14. Literature

Versalovic, James, Carroll, Karen C., Funke, Guido, Jorgensen, James H., Landry, Marie Louise and David W. Warnock (ed). Manual of Clinical Microbiology. 10th Edition. ASM Press, 2011.

Cohen, Jonathan, Powderly, William G, and Steven M Opal. Infectious Diseases, Third Edition. Mosby, 2010.

15. Trademarks and Disclaimers

AltoStar®, RealStar® (altona Diagnostics); ABI Prism® (Applied Biosystems); BD MAX™ (BD); NucliSENS®, EasyMag® (bioMérieux); CFX96™ (Bio-Rad); Universal Transport Medium™, UTM® (Copan); JOE™ (Life Technologies); Maxwell® (Promega); Rotor-Gene®, QIAamp®, QIAsymphony® (QIAGEN); LightCycler® (Roche); VERSANT® (Siemens Healthcare); FAM™, ROX™ (Thermo Fisher Scientific).

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The RealStar® SARS-CoV-2 RT-PCR Kit 1.0 is a CE-marked diagnostic kit according to the European *in vitro* diagnostic directive 98/79/EC.

Product not licensed with Health Canada and not FDA cleared or approved.

altona Diagnostics RealStar® SARS-CoV-2 RT-PCR Kit 1.0 has received Provisional Authorisation from the Health Sciences Authority in Singapore.

Not available in all countries.

16. Explanation of Symbols

Symbol	Explanation
IVD	*In vitro* diagnostic medical device
LOT	Batch code
CAP	Cap color
REF	Catalogue number
CONT	Content
NUM	Number
COMP	Component
GTIN	Global trade item number
	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
i	Note: Information is given to the user that is useful but not essential to the task at hand.
	Version

Notes:

always a drop ahead.

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