Important note:

Changes in Instruction for Use version 2.1 are highlighted.

Instruction for Use

virellaSARS-CoV-2 seqc

real time RT-PCR Kit 2.0

For the simultaneous in vitro detection of RNA of novel coronavirus (SARS-CoV-2) and Subgenus Sarbecovirus (SARS-CoV-1 and SARS-CoV-2), extracted from biological specimens.

REF	**G01128-96**	**G01128-384**	**G01128-768**
Σ	96	384	768

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1 Intended Use

The virellaSARS-CoV-2 seqc real time RT-PCR Kit 2.0 is a screening assay for the simultaneous detection of RNA of novel coronavirus (SARS-CoV-2) and the Subgenus Sarbecovirus (SARS related Betacoronavirus: SARS-CoV-1 and SARS-CoV-2) extracted from biological specimens.

2 Pathogen Information

Coronaviruses (CoV) are a large family of viruses that cause illness ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS). The novel Coronavirus (SARS-CoV-2) is a new strain within the Sarbecoviruses that has been previously identified in humans and causes the pulmonary disease COVID-19.

Coronaviruses are zoonotic, meaning they are transmitted between animals and people. Detailed investigations found that SARS-CoV was transmitted from civet cats to humans and MERS-CoV from dromedary camels to humans. Several known Coronaviruses are circulating in animals that have not yet infected humans.

Common signs of infection include respiratory symptoms, fever, cough, shortness of breath and breathing difficulties. In more severe cases, infection can cause pneumonia, severe acute respiratory syndrome, kidney failure and even death.

Standard recommendations to prevent infection spread include regular hand washing, covering mouth and nose when coughing and sneezing, thoroughly cooking meat and eggs. Avoid close contact with anyone showing symptoms of respiratory illness such as coughing and sneezing.

3 Principle of the Test

The virellaSARS-CoV-2 seqc real time RT-PCR Kit 2.0 contains specific primers and dual-labeled probes for the amplification of RNA (cDNA) of SARS-CoV-2 (both, RdRP gene and S gene, FAM channel) and the RNA (cDNA) of the Subgenus Sarbecoviruses (SARS-CoV-1 and SARS-CoV-2, E gene, Cy5 channel) extracted from biological specimens. Both, E gene and RdRP gene are target sequences of the viral genome recommended by the WHO. The simultaneous detection of 3 target sequences (RdRP gene, S gene and E gene) increases the diagnostic reliability, even in cases of target sequence mutations.

Furthermore, virellaSARS-CoV-2 seqc real time RT-PCR Kit 2.0 contains a Control RNA (Internal Process Control, IPC), which is added during RNA extraction and detected in the same reaction by a HEX-labeled probe.
The Control RNA allows the detection of RT-PCR inhibition and acts as control, that the nucleic acid was isolated from the biological specimen.
Additionally, virellaSARS-CoV-2 seqc real time RT-PCR Kit 2.0 contains an Internal System Control (ISC). The ISC consists of primers and probes for the detection of a house keeping gene (Beta-actin, multi species) in the eluate from a biological specimen. The ISC helps preventing false negative results due to insufficient sample drawing or transport. The amplification of the Beta-actin target sequence is measured in the ROX channel.

4 Package Contents

The reagents supplied are sufficient for 96, 384 or 768 reactions respectively.

Table 1: Components of the virellaSARS-CoV-2 seqc real time RT-PCR Kit 2.0

Label	Lid Colour	Content		
		96	384	768
Reaction Mix	yellow	1 x 1325 µl	4 x 1325 µl	8 x 1325 µl
Enzyme	blue	1 x 19.2 µl	1 x 76.8 µl	2 x 76.8 µl
Positive Control	red	1 x 150 µl	1 x 300 µl	1 x 300 µl
Negative Control	green	1 x 150 µl	1 x 300 µl	1 x 300 µl
Control RNA	colourless	1 x 480 µl	2 x 960 µl	4 x 960 µl

5 Equipment and Reagents to be Supplied by User

- RNA isolation kit (e.g. NukEx Pure RNA/DNA, gerbion Cat. No. G05004 or NukEx Mag RNA/DNA, gerbion Cat. No. G05012)
- Sterile microtubes
- Pipets (adjustable volume)
- Sterile pipet tips with filter
- Table centrifuge
- Vortexer
- Real time PCR instrument
- Optical PCR reaction tubes with lid or optical PCR reaction plate with optical foil
- Optional: Liquid handling system for automation

6 Transport, Storage and Stability

The virellaSARS-CoV-2 seqc real time RT-PCR Kit 2.0 is shipped on dry ice or cool packs. All components must be stored at maximum -18°C in the dark immediately after receipt. Do not use reagents after the date of expiry printed on the package. Up to 20 freeze and thaw cycles are possible. For convenience, opened reagents can be stored at +2-8°C for up to 6 months. Protect kit components from direct sunlight during the complete test run.

7 Warnings and Precautions

Read the Instructions for Use carefully before using the product.
Before first use check the product and its components for:

- Use of this product is limited to personnel specially instructed and trained in the techniques of real time PCR procedures.
- Specimens should always be treated as infectious and/or biohazardous in accordance with safe laboratory procedures.
- Avoid microbial and nuclease (DNase/RNase) contamination of the eluates and the components of the kit.
- Always use DNase/RNase-free disposable pipette tips with aerosol barriers.
- Always wear protective disposable powder-free gloves when handling kit components.
- Use separated and segregated working areas for (1) sample preparation, (2) reaction setup and (3) amplification/detection activities. The workflow in the laboratory should proceed in unidirectional manner.

Always wear disposable gloves in each area and change them before entering a different area.

- Dedicate supplies and equipment to the separate working areas and do not move them from one area to another.
- Store positive and/or potentially positive material separated from all other components of the kit.
- Do not open the reaction tubes/plates post amplification to avoid contamination with amplicons.
- Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations.
- Do not autoclave reaction tubes after the PCR, since this will not degrade the amplified nucleic acid and will bear the risk to contaminate the laboratory area.
- Discard sample and assay waste according to your local safety regulations.

8 Sample Material

Starting material for virellaSARS-CoV-2 seqc real time RT-PCR Kit 2.0 is RNA isolated from biological specimens (e.g. swabs, sputum).

9 Sample Preparation

Commercial kits for RNA isolation such as the following are recommended:

- NukEx Pure RNA/DNA, gerbion Cat. No. G05004
- NukEx Mag RNA/DNA, gerbion Cat. No. G05012

Please follow the instructions for use of the respective extraction kit.

Important:

In addition to the samples always run a ‚water control' in your extraction. Treat this water control analogous to a sample.

Comparing the amplification of the Control RNA in the samples to the amplification of the internal control in the water control will give insights on possible inhibitions of the real time RT-PCR. Furthermore, possible contaminations during RNA extraction will be detectable.

Please note the chapter ‚Control RNA'.

If the real time RT-PCR is not performed immediately, store extracted RNA according to the instructions given by the manufacturer.

10 Control RNA

A Control RNA is supplied and can be used as extraction control or only as inhibition control. This allows the user to control the RNA isolation procedure and to check for possible real time RT-PCR inhibition.

a) Control RNA used as Extraction Control:

Add 5 µl Control RNA per extraction (5 µl x (N+1)). Mix well. Perform the RNA isolation according to the manufacturer's instructions, follow protocol A.
The Control RNA must be added to the Lysis Buffer of the extraction kit.

b) Control RNA used as Internal Control of the real time RT-PCR:

If only inhibition will be checked please follow protocol B.

11 Real time RT-PCR

11.1 Important Points Before Starting:

- Please pay attention to the chapter 7 ‚Warnings and Precautions'.
- Before setting up the real time RT-PCR familiarise yourself with the real time PCR instrument and read the user manual supplied with the instrument.
- The programming of the thermal profile should take place before the RT-PCR set up.
- In every RT-PCR run one Positive Control and one Negative Control should be included.
- Before each use, all reagents should be thawed completely at room temperature, thoroughly mixed and centrifuged very briefly.
- Due to the high viscosity of the Enzyme (blue lid), prewarming at room temperature for 15 min is recommended.

11.2 Procedure

If the Control RNA is used to control both, the real time RT-PCR and the RNA isolation procedure, please follow protocol A. If the Control RNA is solely used to detect possible inhibition of the real time RT-PCR, follow protocol B.

Protocol A

The Control RNA was added during RNA extraction (chapter 10 ‚Control RNA'). In this case, prepare the Master Mix according to Table 2.

The Master Mix contains all of the components needed for the real time RT-PCR except the sample. Prepare a volume of Master Mix for at least one sample more than required, in order to compensate for pipetting inaccuracy.

Table 2:Preparation of the Master Mix (Control RNA was added during RNA extraction)

Volume per Reaction	Volume Master Mix
13.8 µl Reaction Mix	13.8 µl x (N+1)
0.2 µl Enzyme	0.2 µl x (N+1)

Protocol B

The Control RNA is used for the control of the real time RT-PCR only (see chapter 10, Control RNA'). In this case, prepare the Master Mix according to Table 3.

The Master Mix contains all of the components needed for the real time RT-PCR except the sample. Prepare a volume of Master Mix for at least one sample more than required, in order to compensate for pipetting inaccuracy.

Table 3: Preparation of the Master Mix (Control RNA is added directly to the Master Mix)

Volume per Reaction	Volume Master Mix
13.8 µl Reaction Mix	13.8 µl x (N+1)
0.2 µl Enzyme	0.2 µl x (N+1)
0.2 µl Control RNA*	0.2 µl x (N+1)*

*The increase in volume caused by adding the Control RNA is not taken into account when preparing the RT-PCR assay.

Protocol A and B: real time RT-PCR set-up

- Place the number of optical PCR reaction tubes needed into the respective tray of the real time PCR instrument / take an optical PCR reaction plate.
- Pipet **14 µl** of the Master Mix into each optical PCR reaction tube / the optical PCR reaction plate.
- Add **6 µl** of the eluates from the RNA isolation (including the eluate of the water control), the Positive Control and the Negative Control to the corresponding optical PCR reaction tube / the optical PCR reaction plate (Table 4).
- Close the optical PCR reaction tubes / the optical PCR reaction plate immediately after filling in order to reduce the risk of contamination.

Table 4: Preparation of the real time RT-PCR

Component	Volume
Master Mix	14.0 µl
Sample	6.0 µl
Total Volume	20.0 µl

11.3 Instrument Settings

For the real time RT-PCR use the thermal profile shown in Table 5.

Table 5: real time RT-PCR thermal profile

Description	Time	Temperature	Number of Cycles
Reverse Transcription	10 min	45°C	1
Initial Denaturation	5 min	95°C	1
Amplification of cDNA			45
Denaturation	10 sec	95°C	
Annealing and Extension	40 sec Acquisition at the end of this step	60°C	

Dependent on the real time PCR instrument used, further instrument settings have to be adjusted according to Table 6.

Table 6: Overview of the instrument settings required for the virellaSARS-CoV-2 seqc real time RT-PCR 2.0.

Real time PCR Instrument	**Parameter Reaction Mix**	**Detection Channel**	**Notes**		
LightCycler 480II			Colour Compensation Kit SARS-CoV-2 seqc (G070MP2-CC) required		
			Melt Factor	Quant Factor	Max Integration Time (sec)
	RdRP gene / S gene	465-510	1	10	1
	Control RNA (IPC)	533-580	1	10	2
	ISC	533-610	1	10	2
	E gene	618-660	1	10	3
Stratagene Mx3000P / Mx3005P	RdRP gene / S gene	FAM	Gain 8	Reference Dye: None	
	Control RNA (IPC)	HEX	Gain 1		
	ISC	ROX	Gain 1		
	E gene	Cy5	Gain 4		
AriaMx Bio-Rad CFX96	RdRP gene / S gene	FAM	Reference Dye: None		
	Control RNA (IPC)	HEX			
	ISC	ROX			
	E gene	Cy5			
ABI 7500	RdRP gene / S gene	FAM	Option Reference Dye ROX: NO		
	Control RNA (IPC)	JOE			
	ISC	ROX			
	E gene	Cy5			

Real time PCR Instrument	Parameter Reaction Mix	Detection Channel	Notes	
Rotor-Gene Q, Rotor-Gene 3000 Rotor-Gene 6000	RdRP gene / S gene	Green	Gain 5	Outlier Removal NTC Threshold 15%
	Control RNA (IPC)	Yellow	Gain 5	
	ISC	Orange	Gain 5	
	E gene	Red	Gain 5	
Mic qPCR Cycler	RdRP gene / S gene	Green	Gain 8	
	Control RNA (IPC)	Yellow	Gain 10	
	ISC	Orange	Gain 10	
	E gene	Red	Gain 10	

12 Data Analysis

Following results can occur:

Signal/Ct Values				**Interpretation**
FAM Channel RdRP gene S gene	**Cy5 Channel** E gene	**ROX Channel** ISC	**HEX Channel** Control RNA (IPC)	
positive	negative	positive or negative	positive or negative*	**Positive result, the sample contains SARS-CoV-2-RNA.**
positive	**positive**	positive or negative	positive or negative*	**Positive result, the sample contains SARS-CoV-2-RNA.**
negative	**positive**	positive or negative	positive or negative*	**Positive result, the sample contains SARS-CoV-2 RNA or SARS-CoV-1 -RNA*.**
negative	negative	positive	≤ 34	**Negative result, the sample contains no SARS-CoV-2-RNA and no SARS-CoV-1 -RNA*.**
negative	negative	negative	≤ 34	**No diagnostic statement can be made.** Amount or quality of sample material not sufficient.
negative	negative	positive	negative or > 34	**Caution!** The real time RT-PCR is either inhibited or errors occurred while RNA/DNA extraction.
negative	negative	negative	negative or > 34	**Caution!** The real time RT-PCR is either inhibited or errors occurred while RNA/DNA extraction. Amount or quality of sample material not sufficient.

*SARS-CoV-1 infections have not been reported since 2004 (Lit. [5]).

Figure 1, Figure 2, Figure 3 and **Figure 4** show examples for positive and negative real time RT-PCR results.

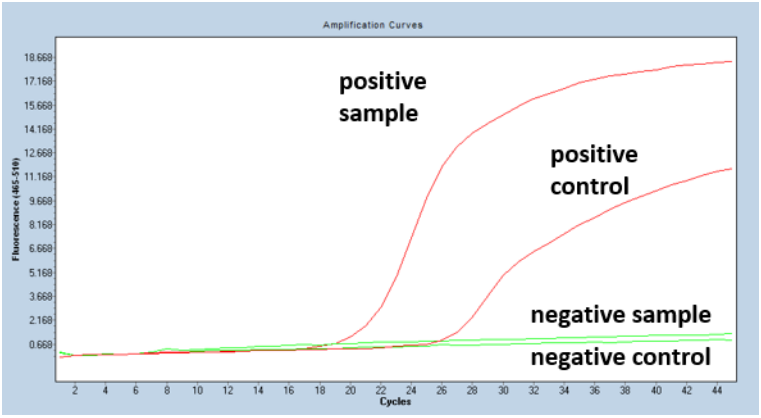

Figure 1: The positive sample shows pathogen specific amplification in the FAM channel (positive sample and positive control), whereas no fluorescence signal is detected in the negative sample or the negative control (LC480 II real time PCR instrument).

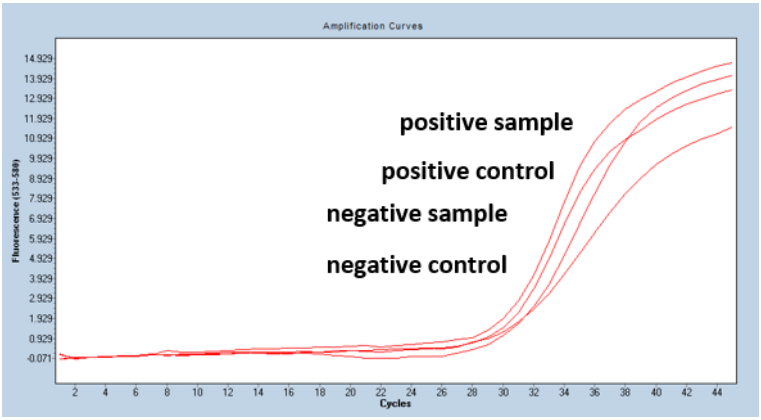

Figure 2: The positive sample, the positive control, the negative control as well as the negative sample show a signal in the Control RNA specific HEX channel (IPC). The amplification signal of the Control RNA in the negative sample shows that the missing signals in the pathogen specific channels FAM and Cy5 are not due to RT-PCR inhibition or failure of RNA isolation, but that the sample is a true negative sample (LC480 II real time PCR instrument).

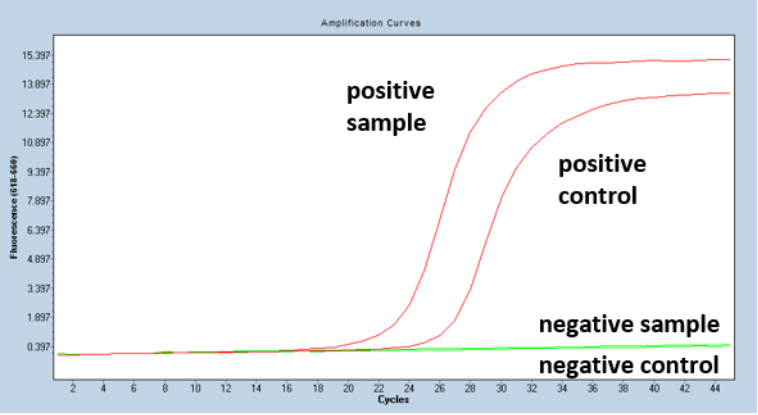

Figure 3: The positive sample shows pathogen specific amplification in the Cy5 channel (positive sample and positive control), whereas no fluorescence signal is detected in the negative sample and the negative control (LC480 II real time PCR instrument).

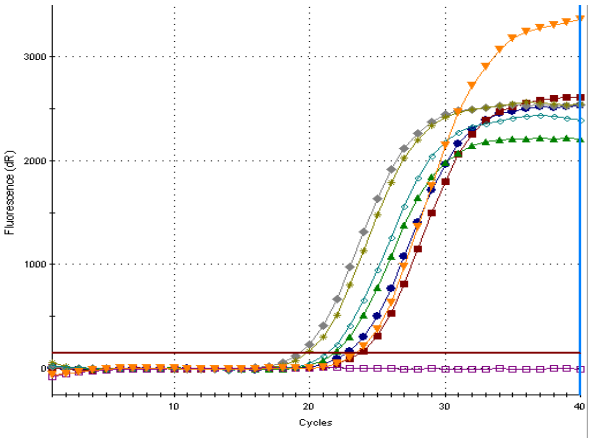

Figure 4: Signals of the amplification of the ISC in the ROX channel. The Figure shows the C_T values of eluates from respiratory swabs after nucleic acid extraction using NukEx Mag RNA/DNA nucleic acid extraction kit (Stratagene Mx3005 P real time PCR instrument).

13 Assay Validation

To increase process safety IPC is included in the Negative Control and Positve Control.

Negative Control

The negative control must show no C_T in the FAM and Cy5 channel. The HEX channel (IPC) in the Negative Control must show a C_T of below 34. Due to the high sensitivity of the virellaSARS-CoV-2 real time RT-PCR 2.0, a weak positive result in the ROX channel (ISC) caused by slight contaminations with human DNA during RT-PCR set up cannot completely be ruled out. This does not affect the validity of the respective run (see also Internal Controls).

Positive Control

All the positive controls must show a positive (i.e. exponential) amplification curve in the different channels FAM, Cy5, ROX and HEX. The positive controls must fall below a C_T 30 of except for the HEX channel, which must show a C_T of below 34. Positive Control includes in vitro transcripts and synthetic DNA of approximately 10^4 copies per reaction for RdRP gene, E gene and ISC.

Internal Controls

The following values for the amplification of the internal controls are valid using gerbion nucleic acid extraction kits NukEx Mag RNA/DNA and NukEx Pure RNA/DNA. All internal controls (ISC and IPC, seqc – sample and extraction quality control) must show a positive (i.e. exponential) amplification curve. The Control RNA (IPC) must fall below a C_T of 34. If the Control RNA is above C_T 34 this points to a purification problem or a strong positive sample that can inhibit the IPC. In the latter case, the assay is valid. It is recommended to perform the extraction of a water control in each run. The IPC in the water control must fall below a C_T of 34. For accurately drawn respiratory swab samples, the ISC shows C_T values from app. 15 to app. 28. A heavily delayed signal of higher than a C_T of 34 indicates a low sample amount. Therefore, false negative results cannot be ruled out. In case of no amplifications neither in the FAM nor in the Cy5 channel, there must be an amplification curve in the ROX channel (ISC) and the HEX (IPC) channel when using eluates of primary samples from multiple species such as mammals and birds.

If other nucleic acid extraction kits are used, the customer must define own cutoffs. In this case the C_T value of the Control RNA (IPC) in an eluate from a sample should not be delayed for more than 4 C_T in comparison to an eluate from an extracted water control.

14 Limitations of the Method

- Strict compliance with the Instruction 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.
- All reagents should be closely monitored for impurity and contamination. Any suspicious reagents should be discarded.
- This assay must not be used on a biological specimen directly. Appropriate nucleic acid extraction methods have to be conducted prior to using this assay.
- The presence of RT-PCR inhibitors may cause false negative or invalid results.

- Potential mutations within the target regions of the SARS-CoV-2 and Betacoronavirus genomes covered by the primers and/or probes used in the kit may result in failure to detect the respective RNA.
- As with any diagnostic test, results of the virellaSARS-CoV-2 seqc real time RT-PCR Kit 2.0 need to be interpreted in consideration of all clinical and laboratory findings.

15 Troubleshooting

The following troubleshooting guide is included to help you with possible problems that may arise when performing a real time RT-PCR. If you have further questions, please do not hesitate to contact our scientists on info@gerbion.com.

No fluorescence signal in the FAM and Cy5 channel of the Positive Control	
The selected channel for analysis does not comply with the protocol	Select the FAM channel for analysis of the SARS-CoV-2 specific amplification, the Cy5 channel for analysis of the Sarbecovirus specific amplification, the HEX channel for the amplification of the Control RNA and the ROX channel for the amplification of the ISC.
Incorrect preparation of the Master Mix	Make sure, the Enzyme is added to the Master Mix (chapter 11).
Incorrect configuration of the real time RT-PCR	Check your work steps and compare with "Procedure" on page 7.
The programming of the thermal profile is incorrect	Compare the thermal profile with the protocol "Instrument Settings" on page 9.
Incorrect storage conditions for one or more kit components or kit expired	Check the storage conditions and the date of expiry printed on the kit label. If necessary, use a new kit and make sure kit components are stored as described in "Transport, Storage and Stability
Weak or no signal of the Control RNA and ISC and simultaneous absence of a signal in the FAM and/or Cy5 channel.	
real time RT-PCR conditions do not comply with the protocol	Check the real time RT-PCR conditions (page 7).
real time RT-PCR inhibited	Make sure that you use an appropriate isolation method (see chapter "Sample Preparation") and follow the manufacturer's instructions. Make sure that the ethanol-containing washing buffers have been completely removed.

sample material not sufficient	Make sure that enough sample material has been applied to the extraction. Use an appropriate isolation method (see chapter "Sample Preparation") and follow the manufacturer's instructions.
RNA loss during isolation process	In case the Control RNA was added before extraction, the lack of an amplification signal can indicate that the RNA isolation was not successful. Make sure that you use an appropriate isolation method (commercial kits are recommended) and stick to the manufacturer's protocol.
Incorrect storage conditions for one or more components or kit expired	Check the storage conditions and the date of expiry printed on the kit label. If necessary, use a new kit and make sure kit components are stored as described in "Transport, Storage and Stability
Detection of a fluorescence signal in the FAM and/or Cy5 channel of the Negative Control	
Contamination during preparation of the real time RT-PCR	Repeat the real time RT-PCR in replicates. If the result is negative in the repetition, the contamination occurred when the samples were pipetted into the optical PCR reaction tubes. Make sure to pipet the Positive Control last and close the optical PCR reaction tube immediately after adding the sample. If the same result occurs, one or more of the kit components might be contaminated. Make sure that work space and instruments are decontaminated regularly. Use a new kit and repeat the real time RT-PCR.
Detection of a fluorescence signal in the ROX channel of the Negative Control	
Contamination with human DNA during preparation of the real time RT-PCR	As long as the ROX channel shows very high Ct values, the contamination is negligible. If the FAM and Cy5 channel are negative in the Negative Control, the PCR is still valid for the detection of SARS-CoV-2.

16 Kit Performance

16.1 Analytical Sensitivity

The limit of detection (LoD) of virellaSARS-CoV-2 seqc real time RT-PCR Kit 2.0 was determined using serial dilutions of synthetic RNA-fragments containing the RdRP gene target sequence, the S gene target sequence and the E gene target sequence in a Stratagene Mx3005 real time PCR instrument. The LoD of virellaSARS-CoV-2 seqc real time RT-PCR 2.0 Kit is $\leq$10 genome copies per reaction each.

16.2 Analytical Specificity

The specificity of the virellaSARS-CoV-2 seqc real time RT-PCR Kit 2.0 was evaluated with different other relevant viruses and bacteria found in clinical samples and basing on in silico analyses.

Results:

The virellaSARS-CoV-2 seqc real time RT-PCR Kit 2.0 showed a positive result for the samples containing SARS-CoV-2 and Sarbecovirus RNA sequences, whereas samples containing other pathogens were reliably tested negative. The results are shown in table 7.

Table 7: Eluted DNA and RNA from bacterial and viral pathogens tested for the determination of the analytical specificity of virellaSARS-CoV-2 seqc real time RT-PCR Kit 2.0.

Eluates with known status	Expected Result E gene **Cy5 channel**	Expected Result RdRP gene / S gene **FAM channel**	**virellaSARS-CoV-2 seqc** E gene **Cy5 channel**	**virellaSARS-CoV-2 seqc** RdRP gene / S gene **FAM channel**
SARS-CoV-2	***positive***	***positive***	***positive***	***positive***
SARS-CoV-1	***positive***	negative	***positive***	negative
MERS-CoV	negative	negative	negative	negative
HCoV-229E	negative	negative	negative	negative
HCoV-OC43	negative	negative	negative	negative
Influenza A H3N2	negative	negative	negative	negative
Influenza A H5N1	negative	negative	negative	negative
Influenzavirus B	negative	negative	negative	negative
Respiratory Syncytial Virus A	negative	negative	negative	negative
Respiratory Syncytial Virus B	negative	negative	negative	negative
Parainfluenzavirus 1	negative	negative	negative	negative
Parainfluenzavirus 2	negative	negative	negative	negative
Parainfluenzavirus 3	negative	negative	negative	negative
Parainfluenzavirus 4	negative	negative	negative	negative
Metapneumovirus	negative	negative	negative	negative
Adenovirus	negative	negative	negative	negative
Rhinoviruses	negative	negative	negative	negative
Enteroviruses	negative	negative	negative	negative
Human Bocavirus	negative	negative	negative	negative
Legionella pneumoniae	negative	negative	negative	negative
Mycoplasma pneumoniae	negative	negative	negative	negative

Mycobacterium tuberculosis complex	negative	negative	negative	negative
Bordetella pertussis	negative	negative	negative	negative
Bordetella parapertussis	negative	negative	negative	negative
S. aureus	negative	negative	negative	negative
MRSA	negative	negative	negative	negative
MSSA	negative	negative	negative	negative
Streptococcus spp.	negative	negative	negative	negative

16.3 Clinical Samples

Positive (50) and negative (153) confirmed samples (oral and nasal swabs) from the pandemic outbreak 2020 in Europe were tested.
The RNA was extracted by using the NukEx Mag RNA/DNA (gerbion Cat. No. G05012) extraction kit on a KingFisher Prime Duo Instrument.
The PCR experiments were performed on a MX3005P Stratagene Cycler. The testing of the confirmed samples with virellaSARS-CoV-2 seqc showed a sensitivity of 100% and a specificity of 100%. None of the samples was inhibited in the real time RT-PCR. For the validation of the virellaSARS-CoV-2 seqc real time RT-PCR Kit 2.0 the eluates of all samples were retested and showed the same results.

	positive samples	negative samples
virellaSARS-CoV-2 seqc positive	50	0
virellaSARS-CoV-2 seqc negative	0	153
	Sensitivity (%)	Specificity (%)
	100	100

16.4 Linear Range

The linear range of the virellaSARS-CoV-2 seqc real time RT-PCR Kit 2.0 was evaluated by analysing logarithmic dilution series of in vitro transcripts and synthetic DNA fragments.

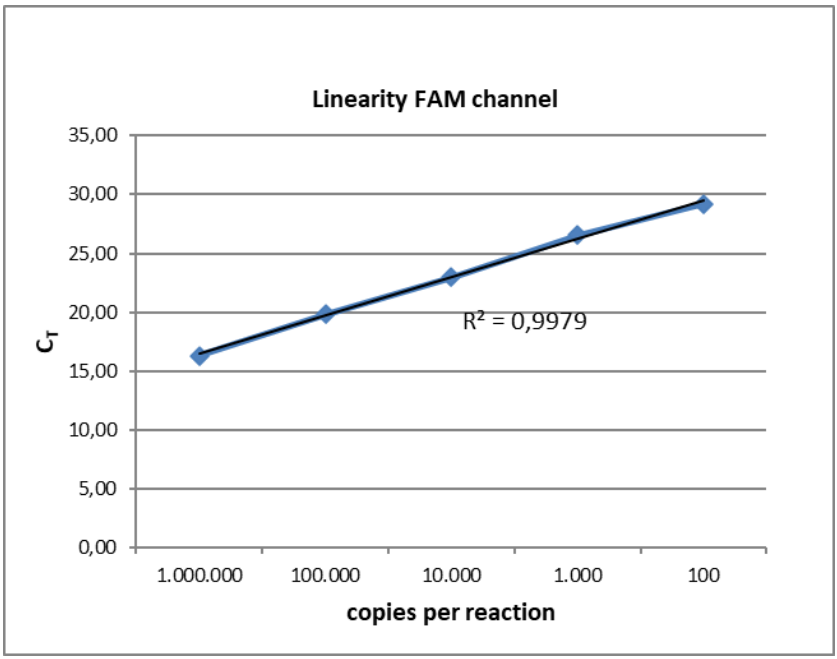

Figure 5: Determination of the linear range of virellaSARS-CoV-2 seqc real time RT-PCR Kit 2.0 in the FAM channel.

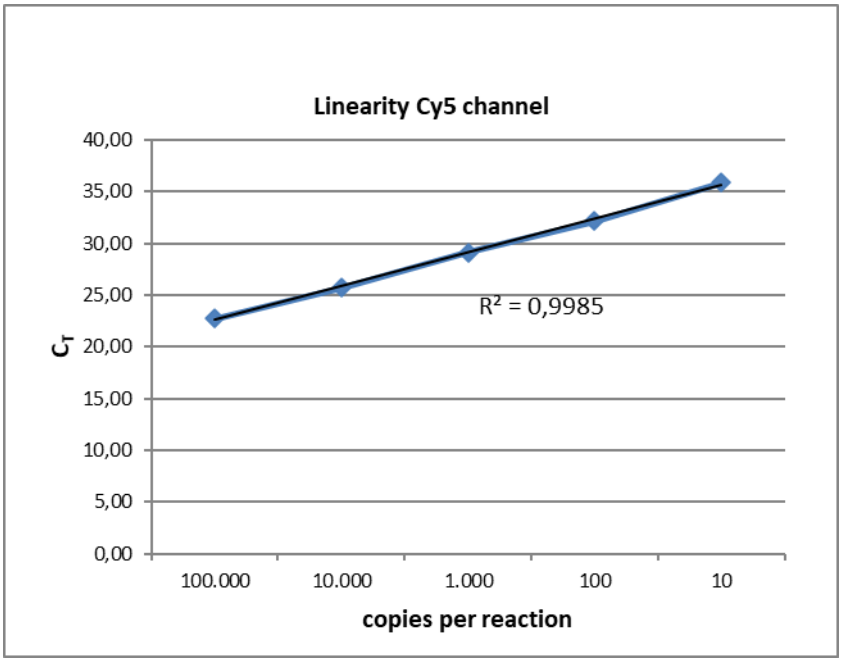

Figure 6: Determination of the linear range of virellaSARS-CoV-2 seqc real time RT-PCR Kit 2.0 in the Cy5 channel.

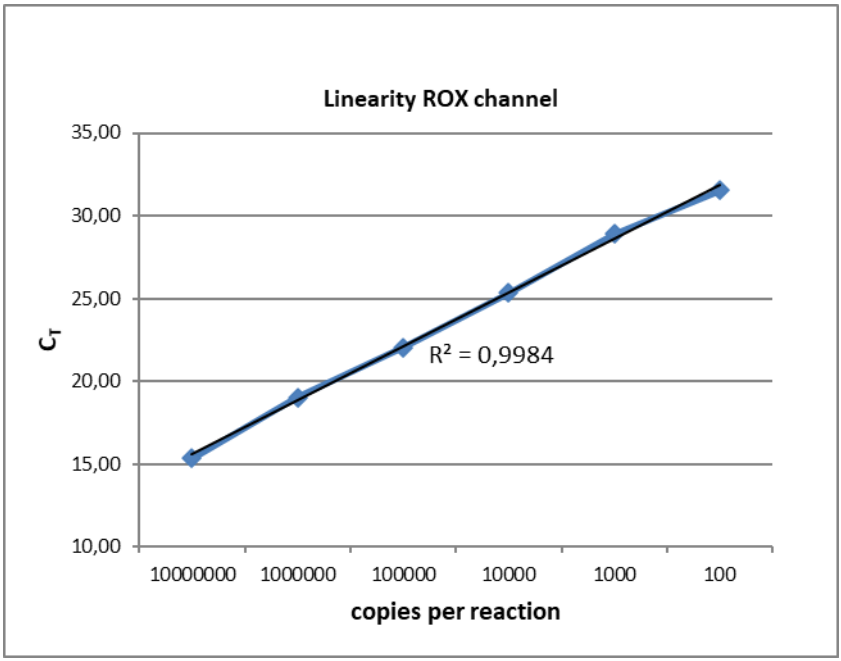

Figure 7: Determination of the linear range of virellaSARS-CoV-2 seqc real time RT-PCR Kit 2.0 in the ROX channel.

16.5 Precision

The precision of the virellaSARS-CoV-2 seqc real time RT-PCR Kit 2.0 was determined as intra-assay variability, inter-assay variability and inter-lot variability.

Variability data are expressed by standard deviation and coefficient of variation. The data are based on quantification analyses of defined concentrations of RdRP gene in vitro transcripts and E gene in vitro transcripts, ISC specific DNA and on the threshold cycle of the Control RNA (IPC).

Table 8: Precision of the virellaSARS-CoV-2 seqc real time RT-PCR Kit.

RdRP gene and S gene (FAM)	copies/µl	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	25	0.23	0.77
Inter-Assay-Variability	25	0.51	1.71
Inter-Lot-Variability	25	0.76	2.56
E gene (Cy5)	copies/µl	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	25	0.27	0.84
Inter-Assay-Variability	25	0.51	1.50
Inter-Lot-Variability	25	0.52	1.61
ISC (ROX)	copies/µl	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	25	0.28	0.90
Inter-Assay-Variability	25	0.40	1.27
Inter-Lot-Variability	25	0.25	0.77
IPC (HEX)	copies/µl	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	25	0.69	2.31
Inter-Assay-Variability	25	0.58	1.91
Inter-Lot-Variability	25	037	1.22

16.6 Diagnostic Sensitivity

The diagnostic sensitivity of real time (RT-) PCR assays is mainly dependent on the DNA/RNA extraction method used to isolate DNA and RNA from various biological specimens. DNA/RNA extraction reagents are not part of the gerbion real time (RT-) PCR kits. gerbion real time (RT-) PCR kits include an extraction control and guidelines for the validation criteria of the extraction control in each reaction. The extraction control indicates inhibition of the real time (RT-) PCR and/or inefficient nucleic acid extraction. It cannot be used as a calibrator.

Therefore, gerbion guarantees the analytical sensitivities and specificities of the real time (RT-) PCR kits, performed with eluted DNA and RNA from reference materials and ring trial samples and with synthetic nucleic acid fragments. gerbion does not guarantee diagnostic sensitivities. If diagnostic sensitivities are mentioned in manuals of gerbion real time (RT-) PCR kits, the data are strictly correlated to a specific nucleic acid extraction method that has been used during the validation of the respective kits and cannot be transferred to other extraction methods.It is the responsibility of the user to qualify the extraction methods used for DNA/RNA isolation from biological samples.

17 Abbreviations and Symbols

RNA	Ribonucleic Acid		Upper limit of temperature
RT-PCR	Reverse Transcription Polymerase Chain Reaction		Manufacturer
REACTION MIX	Reaction Mix		Use by YYYY-MM-DD
ENZYME	Enzyme	LOT	Batch code
CONTROL +	Positive Control	CONT	Content
CONTROL −	Negative Control		Consult instruction for use
CONTROL RNA IPC	Control RNA (IPC)	IVD	*In vitro* diagnostic medical device
REF	Catalog number	CE	European Conformity
	Contains sufficient for <n> tests		

18 Literature

[1] www.who.int/health-topics/coronavirus

[2] Corman et al. Detection of 2019 novel coronavirus (2019-nCoV) by real time RT-PCR. Eurosurveillance, Volume 25, Issue 3, 23/Jan/2020.

[3] www.nature.com/articles/s41564-020-0695-z, 02/March/2020

[4] https://www.ncbi.nlm.nih.gov/research/coronavirus/

[5] https://www.nhs.uk/conditions/sars/