SARS CoV-2 Qualitative

Real Time PCR Kit

For Detection of SARS CoV-2 RNA

50 Tests

The SARS COV-2 Qualitative Real Time PCR Kit is a reagent system, based on real-time PCR technology, for the detection of t RNA.

1. Kit Components

Component	Master A-I	Master A-II	Master B	Internal Control	Positive Control
Number of Vials	1	1	1	1	1
Volume [µl/ Vial]	675	125	55	600	50

2. Storage

- The SARS COV-2 Qualitative Real Time PCR Kit is shipped on 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 us for assistance.
- All components should be stored at -20°C upon arrival.
- Protect Master Mix (A-II) from light.

3. Product Description

The SARS CoV-2 detection is designed for the in vitro quantification of SARS COV-2 genomes. The primers and probe are designed to have 100% homology with the 132 genome sequences available on the GISAID database as of 23 February 2020, some of which were subsequently available on NCBI. The primers and probe sets target the RdRp gene and N Gene which had previously been used in the identification of the SARS coronavirus, however there is no cross reactivity with this or any other coronavirus sequenced thus far. The SARS CoV-2 (2019-nCoV) genomes is designed for the in vitro quantification of SARS COV-2 genomes. The kit is designed to have a broad detection profile. Specifically, the primers represent 100% homology with over 95% of the NCBI database reference sequences available at

the time of design. The dynamics of genetic variation means that new sequence information may become available after the initial design. we periodically reviews the detection profiles of our kits and when required releases new versions. Probes specific for SARS COV-2 RNA are labelled with the fluorophore FAM. The probe specific for the target of the Internal Control (IC) is labelled with the fluorophore HEX. Using probes linked to distinguishable dyes enables the parallel detection of SARS COV-2 specific RNA and Internal Control in the corresponding detector channels of the real-time PCR instrument.

The SARS COV-2 Qualitative Real Time PCR Kit can be used with the following real-time PCR instruments:

- m2000rt (Abbott Diagnostics)
- Mx 3005P™ QPCR System (Stratagene)
- VERSANT™ kPCR Molecular System AD (Siemens)
- ABI Prism® 7500 SDS (Applied Biosystems)
- LightCycler® 480 Instrument II (Roche)
- Rotor-Gene™ 3000/6000 (Corbett Research)
- Rotor-Gene Q 5/6 plex Platform (QIAGEN)

NOTE

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

4. Sample Preparation

Extracted RNA is the starting material for SARS COV-2 Qualitative Real Time PCR Kit. The quality of the extracted RNA has a profound impact on the performance of the entire test system. It has to be ensured that the system used for nucleic acid extraction is compatible with real-time PCR technology.

The following nucleic acid extraction systems and kits are recommended:

- VERSANT™ Molecular System SP (Siemens)
- HighPure® Viral Nucleic Acid Kit (Roche)
- QIAamp® Viral RNA Mini Kit (QIAGEN)

If using a spin column based sample preparation procedure including washing buffers containing ethanol, an additional centrifugation step for 10 min at approximately 17000 x g (~ 13000 rpm), using a new collection tube, prior to the elution of the nucleic acid, is highly recommended.

NOTE

> ⚠ ***The use of carrier RNA is crucial for extraction efficiency and stability of the extracted nucleic acid.***
>
> ⚠ ***Ethanol is a strong inhibitor in real-time PCR. If your sample preparation system is using washing buffers containing ethanol, you need to make sure to eliminate any traces of ethanol prior to elution of the nucleic acid.***

5. Master Mix Setup

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

The SARS COV-2 Qualitative Real Time PCR Kit contains a heterologous Internal Control (IC), which can either be used as a RT-PCR inhibition control or as a control of the sample preparation procedure (nucleic acid extraction) and as a RT-PCR inhibition control.

If the IC is used as a RT-PCR inhibition control, but not as a control for the sample preparation procedure, the Master Mix is set up according to the following pipetting scheme:

Number of Reactions (rxns)	1 SAMPLE
Master A-I	12.5 µl
Master A-II	2.0 µl
Master B	1.0 µl
Internal Control	1.0 µl
Volume Master Mix	**16.5 ul**

If the IC is used as a control for the sample preparation procedure and as a RT-PCR inhibition control, the IC has to be added during the nucleic acid extraction procedure.

No matter which method/system is used for nucleic acid extraction, the IC **must not** be added directly to the specimen. The IC should always be added to the specimen/lysis buffer mixture. The volume of the IC which has to be added depends always and only on the elution volume. It represents 8-10% of the elution volume. For instance, if the nucleic acid is going to be eluted in 60 µl of elution buffer or water,5 - 6 µl of IC per sample must be added to the specimen/lysis buffer mixture.

NOTE

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

• If the IC was added during the sample preparation procedure, the Master Mix is set up according to the following pipetting scheme:

Number of Reactions (rxns)	1 SAMPLE
Master A-I	12.5 µl
Master A-II	2.0 µl
Master B	1.0 µl
Volume Master Mix	**15.5 ul**

6. 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 control (Positive or Negative Control).
- Thoroughly mix the samples and controls with the Master Mix by up and down pipetting.
- Close the 96-well reaction plate with an appropriate optical adhesive film 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).

Reaction Setup		
Master Mix		15 µl
Positive Control		10 µl
Total Volume		**25 µl**

7. Programming the Real-Time PCR Instruments

For basic information regarding the setup and programming of the different real time PCR instruments, please refer to the manual of the respective instrument. For detailed programming instructions regarding the use of the SARS COV-2 Qualitative Real Time PCR Kit. on specific real-time PCR instruments please contact our Technical Support.

7.1 Settings

- Define the following settings:

Settings		
Reaction Volume		25 µl
Ramp Rate		Default
Passive Reference		ROX

7.2 Fluorescent Detectors (Dyes)

- Define the fluorescent detectors (dyes):

Detection	Detector Name	Reporter	Quencher
SARS COV-2 specific RNA	SARS CoV-2	FAM	(None)
Internal Control	IC	HEX	(None)

7.3 Temperature Profile and Dye Acquisition

- Define the temperature profile and dye acquisition:

	Stage	Cycle Repeats	Acquisition	Temperature	Time
Reverse Transcription	Hold	1	-	45 °C	20:00 min
Denaturation	Hold	1	-	95 °C	10:00 min
Amplification	Quantification	50	-	95 °C	15 sec.
			√	60 °C	45 sec.
			-	72 °C	15 sec.

8. Data Analysis

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

For detailed instructions regarding data analysis of the SARS COV-2 RT- PCR 1.0 on different real-time PCR instruments please contact our Technical Support.

9. Interpretation of Results

9.1 Qualitative Analysis

Sample ID	FAM Detection Channels	HEX Detection Channels	Result Interpretation
A	POSITIVE	POSITIVE*	SARS COV-2 specific RNA detected.
B	NEGATIVE	POSITIVE	SARS COV-2 specific RNA not detected. Sample does not contain detectable amounts of SARS COV-2 specific RNA.
C	NEGATIVE	NEGATIVE	RT-PCR inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample.

9.2 Analytical Specificity

The analytical specificity of the SARS COV-2 Qualitative Real Time PCR Kit. is ensured by the thorough selection of the oligonucleotides (primers and probes). The oligo-nucleotides were checked by sequence comparison analysis against public available sequences to ensure that all relevant SARS COV-2 genotypes will be detected.

Over a hundred different SARS COV-2 negative specimens were analyzed with the SARS COV-2 Qualitative Real Time PCR Kit. None of these showed a positive SARS COV-2 specific signal. But all showed a valid IC signal.

In addition, the specificity of the SARS COV-2 Qualitative Real Time PCR Kit. was evaluated by testing a panel of genomic DNA/RNA extracted from other herpesviruses or other pathogens significant in immunocompromised patients.

Table 6: Organisms tested to demonstrate the analytical specificity of the SARS COV-2 Qualitative Real Time PCR Kit.

Organisms	FAM Channel (2019-NCOV)	HEX Channel (Internal Control)
Herpes Simplex Virus 1	Negative	Valid
Herpes Simplex Virus 2	Negative	Valid
Varicella-Zoster Virus	Negative	Valid
Epstein-Barr Virus	Negative	Valid
Human Herpesvirus 6A	Negative	Valid
Human Herpesvirus 6B	Negative	Valid
Human Herpesvirus 7	Negative	Valid
Human Herpesvirus 8	Negative	Valid
Parvovirus B19	Negative	Valid
BK Virus	Negative	Valid
JC Virus	Negative	Valid
Simian Virus 40	Negative	Valid
Hepatitis A Virus	Negative	Valid
Hepatitis B Virus	Negative	Valid
SARS coronavirus	Negative	Valid
MERS coronavirus	Negative	Valid
Coronavirus like virus	Negative	Valid