SPECTRUM

The Creative Approach to Bioscience

SARS COV-2 QUALITATIVE Real Time PCR KIT (DUAL PROBE ASSAY)

REF: 1200 001 100 test

Intended Use

The SARS-CoV-2 Test Kit (Real-time PCR) is an in vitro diagnostic real-time PCR assay intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal/oropharyngeal swabs, anterior/mid-turbinate nasal swabs, nasopharyngeal washes/aspirates or nasal aspirates, and bronchoalveolar lavage (BAL) specimens from individuals suspected of COVID-19 by their healthcare provider

Background

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. Two primers and probe sets target the ORF1ab Gene and N Gene which has 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 Qualitative Detection 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 review the detection profiles of our kits and when required releases new versions. Probes specific for SARS COV-2 RNA are labelled with the fluorophore ROX. The probe specific for the target of the Internal Control (IC) is labelled with the fluorophore FAM 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), StepOne, StepOne Plus and Quant Studio Instruments
- 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's instructions and recommendations.

Precautions and Warnings

Do not ingest or inhalate. In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.

Kit Components

Component	Composition	Number of Vials	Volume [µl/ Vial]
Master A-I	RNA Buffer, dNTP`s, MgCl2, Taq and Taq Antibody	2	1500
Master A-II	Primer and probe mixes for Target -2 (N and O RF1ab) and Interna Control	1	300
Master B	Reverse transcriptase enzyme in enzyme buffer	1	300
Internal Control	Synesthetic RNA construct, serves as Internal Control Target	1	1200
VNAT BUFFER	LYSIS BUFFER FOR Direct PCR test	1	1750
Positive Control	Synesthetic RNA construct, serves as Target for N and ORF1ab genes of SARS CoV-2	1	100

Reagent Storage and Stability

- The SARS COV-2 Qualitative Real Time PCR Kit is shipped on ice. The components of the kit should arrive frozen.

SYMBOLS IN PRODUCT LABELLING

EC REP	Authorised Representative		Use by/Expiration Date
IVD	For in-vitro diagnostic use		CAUTION. Consult instructions for use
LOT	Batch Code/Lot number		
REF	Catalogue Number		Manufactured by
	Consult instructions for use		(Xi) - Irritant
	Temperature Limitation		

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.

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.

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	25 µl
Master A-II	2.5 µl
Master B	2.5 µl
Internal Control	1 µl
Volume Master Mix	31 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	25 µl
Master A-II	2.5 µl
Master B	2.5 µl
Volume Master Mix	30 ul

Reaction Setup (Standard protocol)

• Pipette 30 µl of the Master Mix into each required well of an appropriate optical 96-well reaction plate or an appropriate optical reaction tube.
• Add 20 µl of the sample (eluate from the nucleic acid extraction) or 20 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).

Master Mix	30	µl
Sample or Positive Control	20	µl
Total Volume	50	µl

Reaction Setup (Direct protocol)

• Pipette 30 µl of the Master Mix into each required well of an appropriate optical 96-well reaction plate or an appropriate optical reaction tube.
• Add 15 µl of VNAT buffer to the master mix then add 5ul Saline sample Or 1ul VTM sample AND 1UL IPC TEMPLET
• Thoroughly mix the samples and VNAT buffer 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).

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.

Settings

Define the following settings:

Reaction Volume	50 µl
Ramp Rate	Default

Fluorescent Detectors (Dyes)

Define the fluorescent detectors (dyes):

Detection	Detector Name	Reporter	Quencher
Internal control	IPC	FAM	(None)
SARS COV-2 specific RNA **ORF1ab Gene**	SARS CoV-2 ORF1ab Gene&NGene	ROX	(None)

Temperature Profile and Dye Acquisition

• Define the temperature profile and dye acquisition:

	Stage	Cycle	Acquisition	Temperature	Time
Reverse Transcription	Hold	Repeats	-	50 °C	15:00 min
Denaturation	Hold	1	-	95 °C	01:00 min
Amplification	Quantification	1 40	- v	95 °C 60 °C	10 sec. 30 sec.

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.

Spectrum Diagnostics does not interpret the results of a clinical laboratory procedure ; interpretation of the results is considered the responsibility of qualified medical personnel. All indications of clinical significance are supported by literature references.

Interpretation of Results

Qualitative Analysis

Sample ID	FAM Detection Channels	ROX Detection Channels	Result Interpretation
A	POSITIVE	POSITIVE	SARS COV-2 specific RNA detected.
B	POSITIVE	NEGATIVE	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

Performance Characteristics

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

2-Limit of detection (LOD)

The LOD of the SARS-CoV-2 Test Kit (Real-time PCR) was confirmed using 20 individual extraction replicates consisting of spiked nasopharyngeal swab samples at 500 copies/mL, 200 copies/mL, and 100 copies/ml. The lowest target level at which more than 95% of 20 replicates for nasopharyngeal swab specimens produced positive results was 200 copies/mL.

3-Clinical performance

A study was performed to evaluate the performance of the SARS-CoV-2 Test Kit (Real-time PCR) using nasopharyngeal swab and oropharyngeal swab specimens. A total of 50 frozen positive samples and 50 frozen negative samples that were previously confirmed using an EUA authorized assay were tested with the SARS-CoV-2 Test Kit. All the 50 positive tests were correctly detected by Spectrum SARS-COV-2 kit and None of the 50 negative samples were positive, Therefore, both the positive and negative result agreement were 100% in comparison to the EUA authorized RT-PCR test

Waste Disposal

This product is made to be used in professional laboratories.
Please consult local regulations for a correct waste disposal.
S56: dispose of this material and its container at hazardous or special waste collection point.
S57: use appropriate container to avoid environmental contamination.
S61: avoid release in environment. Refer to special instructions/safety data sheets.

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