SARS-CoV-2 RealFast™ Assay

REF 8-410 / 8-412 Σ 100 / 500 reactions

-30°C -15°C CE IVD

ViennaLab Diagnostics GmbH
Gaudenzdorfer Guertel 43-45
A-1120 Vienna, Austria
Phone: (+43-1) 8120156-0
info@viennalab.com
www.viennalab.com

1. Intended Use

The SARS-CoV-2 RealFast™ Assay is a multiplex reverse transcription real-time PCR (RT-PCR) test for an accurate qualitative one-step detection of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). The presence of SARS-CoV-2 specific RNA in respiratory tract specimens is associated with the Coronavirus Disease 2019 (COVID-19). The kit is intended to test patients suspected of carrying SARS-CoV-2 infection. The Assay Mix targets the *N* and *RdRP/ORF1ab* genes of SARS-CoV-2, and in addition, the human *ACTB* gene to simultaneously control the performance of nucleic acid extraction and RT-PCR reaction.

2. Introduction

SARS-CoV-2 is a positive sense, single stranded RNA virus that emerged in the Wuhan region of China in late 2019. Within two months it spread globally, and COVID-19 was declared a pandemic by the World Health Organization in March 2020. The disease is characterized by mild influenza-like symptoms (e.g. fever, fatigue, sputum production, sore throat, headache, diarrhea and vomiting) in the majority of patients. However, severe to critical disease course with shortness of breath occurs in a small proportion of infected individuals, leading to an overall mortality rate of 0.2 to 1.0%. People over 65 and/or with pre-existing health conditions are especially at risk. Importantly, asymptomatic persons seem to account for approximately 40% to 45% of SARS-CoV-2 infections, but they can also transmit the virus to others. Due to this silent spread, it is essential to test individuals without clinical or subclinical symptoms, who suspectedly have been exposed to SARS-CoV-2, in order to control the pandemic locally, regionally and globally.

3. Kit Contents

			100 / 500 Rxn
RealFast™ **4x RT-PCR Mix**	1 vial	white cap	500 / 2x 1,250 µl
RealFast™ **RTase**	1 vial	yellow cap	100 / 2x 250 µl
SARS-CoV-2 **Assay Mix**	1 vial	purple cap	400 / 2x 1,000 µl
SARS-CoV-2 **Pos. Control**	1 vial	red cap	500 / 500 µl

The kit contains reagents for 100 / 500 reactions in a final volume of 20 µl each.

The RealFast™ RTase is a thermostable Reverse Transcriptase providing rapid and efficient cDNA synthesis. RealFast™ 4x RT-PCR Mix comprises HotStart Taq DNA polymerase and dNTPs in an optimized buffer system. The SARS-CoV-2 Assay Mix consists of primers and dual-labeled hydrolysis probes covering the SARS-CoV-2 *N* and *RdRP/ORF1ab* genes as well as the human *ACTB* gene.

4. Storage and Stability

On arrival, store the SARS-CoV-2 RealFast™ Assay at -30°C to -15°C. The kit withstands up to 10 freeze/thaw cycles with no loss of activity. Avoid prolonged exposure to intense light. If stored correctly, the kit will retain full activity until the expiration date indicated on the label.

5. Product Description

5.1. Principle of the Test

The one-tube test includes a reverse transcription step followed by three simultaneous TaqMan® fluorogenic 5' nuclease assay reactions. The Assay Mix contains gene-specific primer pairs which amplify a 107 nt fragment of the SARS-CoV-2 *RdRP/ORF1ab* gene, a 67 nt fragment of the SARS-CoV-2 *N* gene and a 101 nt fragment of the transcribed human *ACTB* gene, as well as fluorescently labeled hydrolysis probes which hybridize to the target sequences of the amplified fragments. The proximity of the 5'-fluorescent reporter and 3'-quencher dye on intact probes prevents the reporter from fluorescing. During the extension phase of PCR the 5' – 3' exonuclease activity of the Taq DNA polymerase cleaves the 5'-fluorescent reporter from the hybridized probe. The physical separation of the fluorophore from the quencher dye generates a fluorescent signal in real-time, which is proportional to the accumulated PCR product.

Hydrolysis probe	Fluorophore	Channel
N gene (virus)	FAM	520 nm
RdRP/ORF1ab gene (virus)	HEX	556 nm
ACTB gene (human)	Cy5	670 nm

In SARS.CoV-2 positive clinical samples, the virus-specific probes generate fluorescence signals in the FAM and HEX channels. Due to the common presence of human RNA in the extracted specimen, a signal in the Cy5 channel is also expected to emerge. In negative samples, the human *ACTB* gene-specific probe generates a strong fluorescence signal in the Cy5 channel while no or only a baseline signal can be observed in the FAM and HEX channels. For interpretation of alternative signal patterns, please check the table in section 8.

5.2. Real-time PCR Instrument Compatibility

The kit is compatible with various common real-time PCR instruments capable of recording FAM, HEX and Cy5 fluorescence:

- ✓ CFX96™ (Bio-Rad)
- ✓ ABI 7500 Fast (Applied Biosystems®)
- ✓ MIC qPCR Cycler (bms)

The kit is **not suitable** for use with real-time PCR instruments without appropriate fluorescence detection channels.

5.3. Assay Performance Specifications

Determination of **sensitivity** was performed on 58 naso-/oropharyngeal swab samples extracted with the QIAamp Viral RNA Mini Kit (Qiagen) and testing positive for the SARS-CoV-2 sequence with a CE-marked reference kit. The SARS-CoV-2 RealFast™ Assay determined 57/58 samples as positive, which equaled a true positive rate of 98%.

Determination of **specificity** was performed on 118 naso-/oropharyngeal swab samples extracted with the QIAamp Viral RNA Mini Kit (Qiagen) and testing negative for the SARS-CoV-2 sequence with a CE-marked reference kit. The SARS-CoV-2 RealFast™ Assay determined 117/118 samples as negative, which equaled a true negative rate of 99%.

Recommended input volume: 10 µl viral RNA extract.

Limit of detection: 10 SARS-CoV-2 copies per reaction as determined by analyzing log dilution series of synthetic SARS-CoV-2 RNA control spiked into a mixture of SARS-CoV-2 negative RNA extracted from human naso-/oropharyngeal swab specimens. Typically, samples containing 10 SARS-CoV-2 copies (i.e. 1 SARS-CoV-2 copy/µl input RNA) are expected to pass the threshold at Cq 38.5 +/-1.5.

6. Materials Required but not Supplied

Real-time PCR instrument with FAM (520 nm), HEX (556 nm) and Cy5 (670nm) filters, instrument-compatible reaction vessels, disposable powder-free gloves, vortexer, mini-centrifuge for 2.0 mL tubes, tube racks, set of calibrated micropipettes (0.5-1000 µl), sterile tips with aerosol-barrier filter, molecular grade water, viral RNA extraction system, freezer, biohazard waste container.

7. Experimental Protocol

7.1. RNA Extraction

RNA extraction reagents are **not supplied** with the kit.

RNA isolated from naso-/oropharyngeal swabs can be used. Please follow established laboratory safety guidelines *(e.g. https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html)* for handling and processing specimens associated with COVID-19. In order to achieve the maximum analytical sensitivity, setup of the RT-PCR reaction right after the RNA extraction is highly recommended.

7.2. PCR Controls

Always include a **No Template Control** (NTC) in each experiment to confirm absence of potential contamination. It is advisable to run the NTC (use PCR-grade water instead of RNA) in duplicate.

Always include the SARS-CoV-2 **Pos. Control** as positive reference specimen for your unknown samples.

» ***Note:*** *SARS-CoV-2 Pos. Control is a potential source of contamination. Make sure to handle it carefully*. «

7.3. Preparation of SARS-CoV-2 RealFast™ **Master Mix**

Briefly vortex (SARS-CoV-2 Assay Mix) or gently mix by inverting (RealFast™ 4x RT-PCR Mix and RealFast™ RTase) after thawing. Then briefly centrifuge all reagents. Set up RT-PCR on ice or cooling block. Prepare sufficient **Master Mix** for all your reactions (N samples + controls) with an excess of up to 25% to compensate for pipetting inaccuracies:

Component	per reaction	e.g. 16+4 reactions
SARS-CoV-2 Assay Mix	4 µl	80 µl
RealFast™ 4x RT-PCR Mix	5 µl	100 µl
RealFast™ RTase	1 µl	20 µl
Master Mix	**10 µl**	200 µl

Dispense **10 µl Master Mix** into each well. Add **10 µl RNA extract,** nuclease-free water (**NTC**) or SARS-CoV-2 **Pos. Control** to reach a final reaction volume of 20 µl.

To minimize risk of contamination, always pipette templates in the following order: first NTC, then samples, last positive control. Immediately close reaction vessels.

» ***Note:*** *Avoid creating bubbles in the final reaction mix and avoid touching the optical surface of the cap or sealing film without gloves. Both may interfere with fluorescence measurements. Centrifuge briefly if needed.* «

7.4. RT-PCR Program

Program the real-time PCR instrument according to the manufacturer's instructions for RT-PCR experiments. Place the samples into the thermal cycler and run the following program:

Cycles	Temp	Time	Steps
1	50°C	10 min	Reverse transcription
1	95°C	3 min	Polymerase activation and RTase inactivation
45	95°C	15 sec	Denaturation
	57°C	30 sec	Annealing/Extension – **Data acquisition** in FAM, HEX and Cy5 channels

8. Data Analysis / Interpretation of Results

Presence of SARS-CoV-2 sequence in each sample is determined by signals recorded in the **FAM** and **HEX channels**. The presence of human RNA in the input material is determined by signal recorded in the **Cy5 channel**.

Controls / Samples	Amplification in channel			Interpretation
	FAM ***N gene***	**HEX** ***RdRP/ORF1ab gene***	**Cy5** ***ACTB gene***	
No Template Control	NO	NO	NO	---
SARS-CoV-2 Pos. Control	**YES**	**YES**	**YES**	---
Sample 1	**YES**	**YES**	**YES**/NO**	Targeted SARS-CoV-2 sequences are **present**
Sample 2*	**YES**	NO	**YES**/NO**	Targeted SARS-CoV-2 sequence is **present**
Sample 3*	NO	**YES**	**YES**/NO**	Targeted SARS-CoV-2 sequence is **present**
Sample 4	NO	NO	**YES**	Targeted SARS-CoV-2 sequences are **absent**
Sample 5	NO	NO	NO	Repeat test***

* Signal for only one of the two SARS-CoV-2 specific targets might be due to a low number of virus copies close to the limit of detection in the sample or due to mutation(s) affecting the primer/probe binding sites.

** Detection of human *ACTB* is not required for demonstrating the presence of SARS-CoV-2 sequence in the sample. High viral RNA load can suppress or eliminate the signal of the control target.

*** RNA extraction and/or RT-PCR failure.

Some instrument software needs manual threshold settings for accurate signal calling.

Recommendations for Threshold Settings (C_q): Set threshold value for the FAM, HEX and Cy5 channels just above the background fluorescent signal generated by the No Template Control (FAM-HEX-Cy5 negative wells).

In case signals are in the range of the limit of detection (Cq 38.5 +/-1.5), we recommend confirmation by re-testing the sample in triplicate. Samples crossing the threshold line beyond Cq 40 represent inconclusive results, which should be considered as negative.

To analyze acquired data, please follow your instrument software instructions.

9. Warnings and Precautions

- For *in vitro* diagnostics use only.
- Always use disposable powder-free gloves and wear suitable lab coat when handling specimens and reagents.
- Perform reaction setup in an RNase-free area separate from nucleic acid preparation and PCR product analysis.
- Use pipettes dedicated for PCR setup only, use aerosol-guarded pipette tips.
- Use instrument-compatible reaction vessels with optically clear caps or sealers.
- Do not mix reagents from different lots.
- Do not use expired kits or kit components.